DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME SUBSTITUTED SULPHONYL UREA AND GUANIDINE DERIVATIVES AS HYPOGLYCEMIC AGENTS

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

by

Mr. Ishan I Panchal

[Enrollment No: 119997290032]

under supervision of

Dr. Dhrubo Jyoti Sen

GUJARAT TECHNOLOGICAL UNIVERSITY

AHMEDABAD

April-2018

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ABSTRACT

Diabetes mellitus is a major degenerative disease associated with a group of disorders of carbohydrate metabolism results from the body's failure to produce insulin in type 1 and insulin resistance in type 2 diabetes mellitus through altered secretion, decreased insulin activity known as hyperglycemia. There is a direct relationship between hyperglycemia and long-term complications such as, retinopathy, nephropathy and neuropathy like micro and macrovascular concerns. Search for new innocent anti-diabetic agents are still a challenge for medicinal chemists. The detailed study of literature review and study, we have decided to design and synthesis of novel antidiabetic agents with the help of the Crystal structure of the pancreatic ATP-sensitive K^+ channel SUR1/Kir6.2 complexes with ATP and glibenclamide (PDB ID: 5TWV) was imported. Docking, screening and post-analysis of the designed compounds was done using iGEMDOCK program with the protein target 5TWV. The novelty of synthesize compounds was checked by Sci Finder report. All the synthesize compounds were characterized by melting points, TLC, IR spectroscopy, Mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR. The synthesized compounds were proposed for biological evaluation by most relevant animal models like alloxan (150 mg/dl, intraperitonial) induced diabetic animal model for in-vivo studies.

Keywords: Diabetes mellitus, glibenclamide, iGEMDOCK, alloxan

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DEDICATED TO MY PARENTS
LOVING BROTHER KUSHAL,
BELOVED WIFE KOMAL, LITTLE PRINCE NAKSH
FAMILY MEMBERS
& THE SUPREME GOD...

Table of Content

List of Abbreviations

Abbreviations	Full form
DM	Diabetes mellitus
BCE	Before Common Era
HDL	High density lipoprotein
TZD	Thiazolidinediones
DPP-4	Dipeptidyl-peptidase-4 inhibitors
GLP-1	Glucagon-like peptide-1 agonist
SGLT2	Sodium glucose cotransporter 2 inhibitors
DNA	Deoxyrebo nucleic acid
AR	Aldose reductase
NADPH	Nicotinamide adenine dinucleotide phosphate
IP3	Inositol 1, 4, 5-trisphosphate
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
ANOVA	Analysis of variances
CMC	Carboxy methyl cellulose
DC	Diabetic control
IR	Infrared spectra
NMR	Nuclear magnetic resonance
KBr	Potassium bromide
R_f	Retardation factor
MP	Melting point
$FeCl_3$	Ferrous chloride
PSA	Polar surface area
MW	Molecular weight
RMSD	Root Mean Square Deviation
PDB	Protein data bank
SUR	Sulphonylureas receptor
TLC	Thin layer chromatography

List of Symbols

Symbol	Meaning
µg	Microgram
g	Gram
mg	Milligram
Cm	Centimeter
T	Time
Min	Minutes
Hr	Hour
Δ	Heat

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CHAPTER 1

Introduction

1.1 Definitions of problem

The world is facing an epidemic of diabetes mellitus. Diabetes is a set of metabolic illnesses characterized by hyperglycemia resulting from deficiencies in insulin secretion, insulin action, or both. Prediabetes is an impaired glucose tolerance (IGT) or reduced fasting glucose (IFG) condition in which blood glucose level are higher than normal, but not acceptable to be classified as type 2 diabetes [1]. These glucometabolic deviations are connected with cardiovascular morbidity and mortality. Prediabetes characterizes an intermediate phase of an altered glucose metabolism between normal glucose levels and diabetes. Currently, more than 250 million people in the world have diabetes and it is predicted that this number will double in a little over 20 years [2–3].

Diabetes mellitus is a long-lasting endocrine syndrome, categorized by hyperglycaemia resulting from an absolute insulin shortage. It is classified by either type 1 or type 2 diabetes. The origin is either diminished insulin secretion or impaired insulin action or both. [4] DM is possibly one of the oldest diseases known to man. It was first reported in an Egyptian manuscript about 3000 years ago [5]. Evidence from many scientific trials indicates that type 2 diabetes can be delayed or prevented in the at-risk group through existence changes, such as dietary changes, physical activity and weight loss [6]. The pathophysiology of type 1 diabetes derives from the autoimmune destruction of insulin-secreting pancreatic β-cells, resulting in insulin deficiency and subsequent hyperglycaemia. Type 1 diabetes accounts for about 10-15% of all diabetics. Type 2 diabetes is characterized by abnormal insulin secretion due to peripheral resistance and accounts for 85-90% of all persons with diabetes. While type 1 diabetes usually manifests itself in juvenile or adolescence and type 2 diabetes at a later phase, clinical attendance and development vary significantly and some patients might not be clearly classified as having either type 1 or 2 firstly. In the uncontrolled state, both types of diabetes are

characterized by increased hepatic glucose production and decreased glucose uptake in the muscles and adipose tissue. Patients with type 1 diabetes are at risk of severe lipolysis foremost to diabetic ketoacidosis. The remaining insulin movement in type 2 diabetes usually inhibits lipolysis and ketone production such that these patients are less likely to develop ketoacidosis but are more probable to develop a hyperosmolar, non-ketotic state.

Throughout the world the occurrence and prevalence of diabetes continues to rise due to both an increasing incidence of type 1 diabetes in children, and of type II diabetes due to lifestyle deviations particularly in developing nations. Globally, as of 2011, an estimated 366 million people had DM, with type 2 making up about 90% of the cases [7, 8]. The number of people with type 2 DM is increasing in every nation with 80% of people with DM living in low- and middle-income countries. Physical exercise entails multiple physiological and psychological assistances for the diabetic patient. In type 1 diabetes, physical workout theaters an essential role in both physical and mental growth.

1.2 History of DMs [9]

1500 BCE: DMs are first described in an Egyptian manuscript as “too great emptying of the urine.” believed to be of type 1 DMs and it was known a “madhumeha” or “honey urine” by Indian physicians.

230 BCE: "Diabetes a Greek Apollonius of Memphis. The DMs were rare during the period of Roman Empire; Galen had seen only 2 patients in his career.

1st century: The Greek physician ‘Aretaeus of Cappadocia’ noticed the maximal amount of urine, excreted via kidneys named as “diabetes”.

400-500 AD: Indian Practioner, Sushrutha & Co identified T1, T2 DMs with separate conditions, and Type 1 in youth and Type 2 in overweight.

980–1037: In medieval Persia, Avicenna had proposed a wide note on DMs in “The Canon of Medicine”, "mentioning the abnormal of appetite and sexual function collapse,” with ‘the sweet taste of urine’. He recognized primary and secondary DMs, detailed diabetic gangrene, and he gave management by ‘Lupine, Trigonella (Fenugreek) mixture, this treatment is still in practice.

Late 1700s: Britain John Roll termed as "mellitus" or "from honey".

1776: Matthew Dobson realized the 'sweet taste due to a kind of sugar' in urine and blood.

1869: P. Langerhans discovered the islets of Langerhans and recognized key cells in the pancreas, which produce the main material which controls FBG limits.

The Chinese, Japanese and Korean noted the sweetness of urine and words for diabetes as "sugar urine disease".

1921-1922: Frederick Banting and Charles Best showed the actual treatment with insulin in 1921 and 1922.

1922: The metabolism wasn't clarified till 1921, when Sir FG. Banting & CH. Best frequent experimental works of Von Mering and Minkowski; diabetes induced by an extract from the pancreatic islets to vigorous dogs. Banting, Best, and colleagues (Chemist Collip) studied on to purify insulin from bovine pancreases (University of Toronto). The first patient was treated with insulin. Banting and laboratory director John MacLeod received the Nobel Prize in Physiology of Medicine (1923). The chemical structure first discovered by Sanger.

November 14th: So that Bantings birthday November 14th is honored by World diabetes day.

1995: Sanger's group in Cambridge identified the amino acid sequence of insulin, which consists of A (21 AA residues) and B (30AA residues) peptide chains

1.3 Prevalence of diabetes in India

India had more diabetics than any other country in the world, according to the International Diabetes Foundation. While, the country has now been beaten to the top spot by China. India currently has 61.3 million diabetics, a figure that is projected to increase to 103 million by 2030. [10] Closely 1 million Indians die due to diabetes every year. India is home to 69.1 million people with DM and is estimated to have the second highest number of cases of DM in the world after China in 2015. [11]

According to the Indian Heart Association, India is expected to be home to 109 million individuals with diabetes by 2035. A study by the American Diabetes Association reports that India will see the highest increase in people identified with diabetes by 2030.

The high frequency is attributed to a combination of genetic susceptibility plus adoption of a high-calorie intake, low activity lifestyle. It is growing in India's middle class.

1.4 Type 2 diabetes

Researchers do not fully understand why some people suffering from prediabetic and type 2 diabetes and others do not have any complications. It is clear that certain factors increase the risk, however, including

Weight: The more fatty tissue, the more resistant your cells become to insulin.

Inactivity: Physical activity helps you control your weight, uses up glucose as energy and makes your cells more sensitive to insulin.

Family history: Your risk increases if a parent or sibling has type 2 diabetes.

Age: This may be because you tend to exercise less, lose muscle mass and gain weight. Type 2 diabetes is also growing among children, adolescents and younger adults.

Gestational diabetes: Gestational diabetes develops in pregnant women. If mother gave birth to a baby weighing more than 4 kg.

Polycystic ovary syndrome: For women, having polycystic ovary syndrome, a common condition categorized by irregular menstrual periods, excess hair progress and obesity increases the risk of diabetes.

High blood pressure: Having blood pressure over 140/90 mmhg of mercury is linked to an amplified risk of type 2 diabetes.

Abnormal cholesterol and triglyceride levels: If anyone has low levels of high density lipoprotein (HDL), cholesterol, your risk of type 2 diabetes is higher. Triglycerides are another type of fat carried in the blood. People with high levels of triglycerides have an increased danger of type 2 diabetes.

1.5 Gestational diabetes

Pregnant women can develop gestational diabetes, but some women are at greater risk than are others.

1.5.1 Risk factors for gestational diabetes

1.5.1.1 Age: Women older than age 25 are at increased risk.

1.5.1.2 Family or personal history: Risk increases if anyone have prediabetic a precursor to type 2 diabetes or if a close family member, such as a parent, has type 2 diabetes. You are also at greater risk if someone had gestational diabetes during an earlier pregnancy.

1.5.1.3 Weight: Being overweight before pregnancy increases risk.

1.6 Various classes of hypoglycemic agents

In TABLE 1.1 various types of hypoglycemic agents are mentioned. TABLE 1.2 First and second generation sulphonylureas are given.

TABLE 1.1 Classification of hypoglycemic agents

Class	Generic name	Mechanism of action	Adverse effects
Sulphonylureas	Gliclazide Gliclazide Glimepiride Glyburide	Stimulate the pancreas to produce more insulin	Hypoglycemia
Meglitinides	Nateglinide Repaglinide	Stimulate the pancreas to produce more insulin	Hypoglycemia
Biguanides	Metformine	Reduce the production of glucose by the liver	Diarrhea, metallic aftertaste, nausea
Thiazolidinediones (TZD)	Pioglitazone Rosiglitazone	Increase insulin sensitivity of the body cells and reduce the production of glucose by the liver	Swelling due to water retention, weight gain Pioglitazone: increased risk of bladder cancer Rosiglitazone: increased risk of non-fatal heart attack
Alpha-glucosidases inhibitor	Acarbose	Slow the absorption of carbohydrates (sugar) ingested	Bloating and flatulence
Dipeptidyl-peptidase-4 (DPP-4) inhibitors	Linagliptine Saxagliptine Sitagliptine Alogliptine	Intensify the effect of intestinal hormones involved in the control of blood sugar	Pharyngitis, headache
Glucagon-like peptide-1 (GLP-1) agonist	Exenatide . Liraglutide Dulaglutide	Mimic the effect of certain intestinal hormones involved in the control of blood sugar	Nausea, diarrhea, vomiting
Sodium glucose cotransporter 2 (SGLT2) inhibitors	Canaglifozine	Help eliminate glucose in the urine	Genital and urinary infections, more frequent urination

1.7 Mechanisms of Sulfonylurea Hypoglycemia

The Sulphonylureas produce hypoglycemic actions by two mechanisms that can be broadly classified as pancreatic and extra-pancreatic:

1.7.1 Pancreatic Mechanism:

All Sulphonylureas hypoglycemic inhibits the efflux of K+ (K+ channel blockers) from pancreatic ß-cells via a Sulphonylureas receptor which may be strictly linked to an ATP-sensitive K^+ channel. The inhibition of efflux of K+ clues to depolarization of the ß-cell membrane and, as a status, voltage-dependent Ca^{+2} channels on the ß-cell membrane then open to permit entry of Ca^{+2}. The resultant increased necessary of Ca^{+2} to calmodulin

results in activation of kinases related to endocrine secretory granules thereby indorsing the exocytosis of insulin-containing secretory granules.

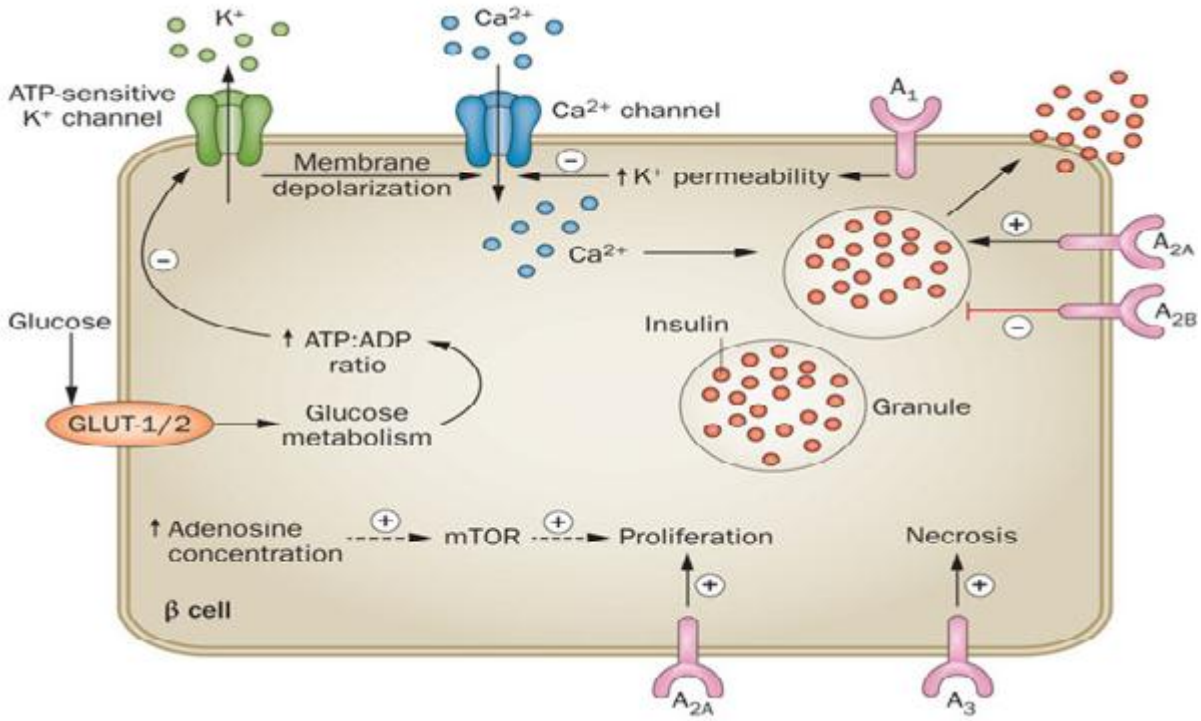
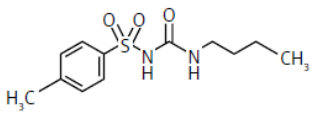
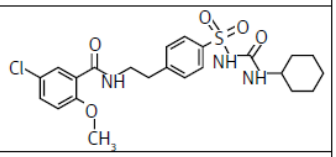
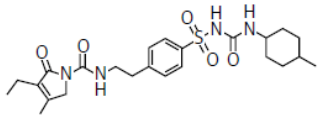
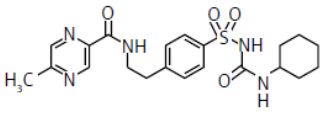
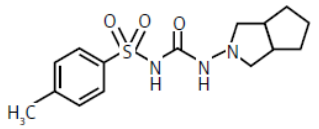
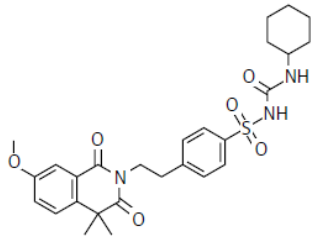


FIGURE 1.1 Pancreatic Mechanism of sulphonylurea

1.7.2. Extra-Pancreatic Mechanisms:

The Sulphonylureas also reduce serum glucagon levels, possibly paying for its hypoglycemic effects. The precise mechanism by which this occurs remains unclear, but may result from indirect reticence due to enhanced discharge of both somatostatin and insulin. Sulphonylureas may also potentiate insulin action at board, tissues drug dependent characteristic.

TABLE 1.2 First and second generation Sulphonylureas [12]

Molecules	Gen.	Dose [mg]	Duration of action* T1/2	Activity of metabolites T1/2	Elimination	Structure
Tolbutamide	I	500–2000	Short 4.5 to 6.5 h	Inactive	Urine ≈ 100%	
Glibenclamide	II	2.5–15	Intermediate to long 5 to 7 h	Active 10 h	Bile ≈ 50%	
Glimepiride	II	1–6	Intermediate 5 to 8 h	Active 3 to 6 h	Urine ≈ 80%	
Glipizide	II	2.5–20	Short to intermediate 2 to 4 h	Inactive	Urine ≈ 70%	
Gliclazide	II	40–320	Intermediate 10 h	Inactive	Urine ≈ 65%	
Gliquidone	II	15–180	Short to intermediate 3 to 4 h	Inactive	Bile ≈ 95%	

**Short duration of activity means < 12 h, intermediate 12–24 h, long over 24 h.*

1.8 Insulin

1.8.1 Chemistry and biosynthesis

It is an endocrine hormone released from β cells of the pancreas, obtained from biological origin and classified as rapid acting, short acting, intermediate acting or long acting. In 1992, insulin was introduced for clinical use before each main meal and one injection in the night, usually at 1 a.m. insulin is a small protein with a molecular weight of 5800. It contains 51 amino acids arranged in two chains A and B linked by disulphide bonds [13-16].

1.8.2 Pharmacodynamics of Insulin

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by motivating several enzymatic reactions that start at the insulin receptors. The stimulus of an intrinsic tyrosine kinase of the insulin receptor results in an increase in skin phosphorylation that consequently increases the membrane absorptivity to glucose through a complicated cascade of intracellular events and by inhibiting hepatic glucose production. [17]

1.8.2.1 Distribution:

Once insulin is released from the pancreas it is quickly distributed throughout the extracellular fluids, with no plasma protein binding.

1.8.2.2 Metabolism:

Insulin metabolism occurs mainly in the liver and, to a much lesser extent, in the kidneys and muscle tissues. The enzyme insulin glutathionetranshydrogense cuts the intermolecular disulfide bonds holding the A and B chains. Insulin has a short half-life (about 5-6 minutes), and 50 % of circulating insulin is neutralized by the liver with each cycle.

1.8.2.3 Excretion:

Insulin is filtered through the kidney's glomeruli and almost completely (98%) reabsorbed in the proximal tubules back into rotation for further schedules and/or metabolism. In normal patients, only 2% of the clean dose is expelled unchanged in urine. Renal impairment marks the rate of insulin fading from circulation.

1.8.3 Advantages and disadvantages of Insulin

Insulin therapy is recommended only when diet or oral hypoglycemic fails to switch blood glucose levels and in case of postpancreatectomy. Insulin analogues are beneficial with low risk of hypoglycemia, particularly nocturnal hypoglycemia. Drawbacks of insulin therapy are like local pain, inconvenience of multiple injections, insulin edema, lipohypertropy, insulin allergy, resistance and above all of this are weight gain [18-20].

1.8.4 Actions and mechanism of action

Insulin facilitates glucose entry into adipose tissues, muscles, and liver by inspiring several enzymatic replies that start at the insulin receptors. The stimulus of an intrinsic tyrosine kinase of the insulin receptor results in an upsurge in membrane phosphorylation that consequently increases the membrane absorptivity to glucose through a complicated force of intracellular events.

1.8.5 Insulin resistance

Insulin resistance underlies adverse metabolic deviations, like the concentrations of insulin, glucose, lipoproteins, lipids, blood pressure, and other cardiovascular diseases. Resistance to insulin therapy ripens in both types of diabetes, however, it is uncommonly with type 1 and if it occurs, resistance can be prompted by either immune or nonimmune factors.

The resistance to insulin is a tissue insensitivity hormone demonstrated as either a decrease in the quantity of insulin receptors or a decrease in insulin affinity to its receptors. Insulin resistance is further classified as either acute or chronic resistance. While severe resistance grows in patients exposed to poisons, surgical trauma, or emotional disorders, chronic resistance is immunological in nature and results after the creation of antibodies to insulin. Resistance occurs frequently in patients with insulin treatment after a period of exclusion.

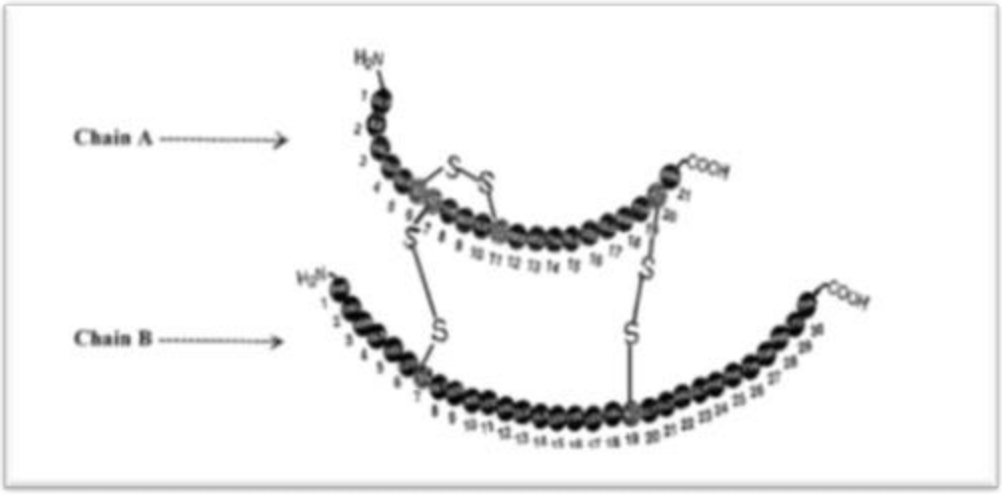


FIGURE 1.2 Structure of Insulin

1.8.6 Pharmaceutical insulin preparations

Injection is recognized routes for insulin administration. While the peptide nature of insulin explains the preclusion of its oral use, numerous oral insulin preparations are under clinical trials to assess effectiveness in providing glycemic regulator for diabetic patients. In addition to efforts to administer insulin orally and by inhalation, several other new routes for distribution are still under study, counting intra peritoneal delivery devices, implantable pellets, closed-loop artificial pancreas, gene therapy, and islet cells and pancreatic additional.

Insulin is commercially exists for subcutaneous, intravenous, and intramuscular use. Insulin preparations have evolved from those produced from animal class to human insulin preparations formed by recombinant DNA technology. Although animal and human insulin preparations do not pointedly differ in doings, increased risk of causing an allergic reaction poses a concern.

Insulin products are classified as rapid, intermediate, or long acting according to their onset and duration of action. The onset and duration of action of insulin products directly relate to the preparation's zinc content. Products containing a low amount of zinc, such as regular insulin, generally act faster and have a short duration of action, while those holding a high amount of zinc, such as ultralente insulin, have a slower onset but long duration of action.

1.8.6.1 Rapid-acting insulin preparations

Insulin products intended to provide rapid action which is prepared in either water for injection or in a phosphate buffer solution containing minute amounts of zinc chloride (0.01- 0.04 mg/100U). Rapid-acting insulin preparations contain regular, lispro, insulin aspart, and glulisine insulins.

1.8.6.2 Regular insulin

Regular insulin is a solution of insulin in either water for injection or phosphate buffer containing minute quantities of zinc chloride (0.01-0.04 mg/100 Units). Zinc ions form multiplexes with insulin and agree the development of insulin hexamer. At the injection site, the hexamer dissociates into dimers and further to monomers that rapidly verbose into circulation and give the rapid onset of action.

1.8.6.3 Insulin lispro

Insulin lispro is the first human insulin analog produced by recombinant DNA technology through site-directed mutation. Lispro insulin has the amino acids 28 and 29 of the B chain switched to become lyspro in its place of the prolys configuration existing in regular human insulin. Insulin lispro differs from regular insulin by the asset of its capacity to dissociate faster into monomer in the subcutaneous tissues. The inversion of B28-pro and B29-lys confers a conformational change in insulin structure that disfavors the establishment of dimers or hexamers and favors the formation of monomer; accordingly, lispro has a faster onset of action than regular insulin.

1.9 Polyol pathway

The mechanism involved in sugar cataract formation is the metabolic imbalance of glucose through the polyol pathway in diabetic patients. He polyol pathway involves two enzymes: aldose reductase and sorbitol dehydrogenase. AR is a member of aldo-ketoreductase family and the first and the key enzyme of the polyol pathway. It reduces glucose to sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is then metabolized to fructose by SDH using NAD+ as a cofactor [21].

Aldose reductase sorbitol and myoinositol are supposed to play a role in the osmoregulation of the kidney [22]. Feeding of NADPH by AR results in the reduction of the levels of NADPH. This NADPH also acts as a cofactor for glutathione reductase,

which reduces oxidized glutathione into summary glutathione . Excess sorbitol is oxidated to fructose. The flux of glucose finished the polyol pathway would increase Advance Glycation end products (AGE) formation. AGES, as well as binding of AGE to their receptors, are recognized to cause oxidative strain.

1.10 Inositol 1, 4, 5-trisphosphate pathway

Inositol 1, 4, 5-trisphosphate (IP3) and calcium (Ca^{2+}) have been my scientific companions over the last 25 years. It has a relationship with these two messengers has approved through two distinct phases. Firstly, there was the work that led up to the discovery that IP3 was a Ca^{2+}mobilizing second messenger. The second phase was categorized by the interest of finding that this IP3/Ca2+ signaling system was a key regulator of numerous changed cellular control mechanisms.

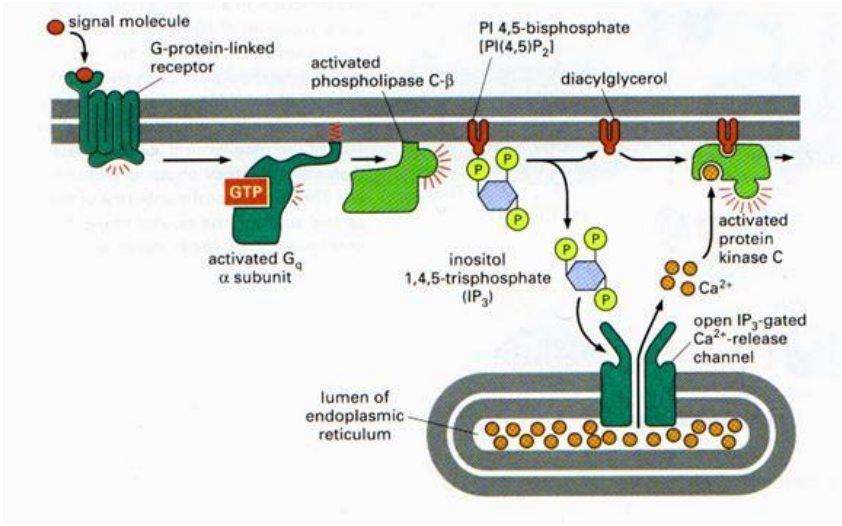


FIGURE 1.3: Inositol 1, 4, 5-trisphosphate pathway

CHAPTER 3

Materials and Methods

3.1 Materials

All the chemicals & reagents were collected from the LR grade from Sigma Alderich, Merck, Chemco, and Acros organics. The reactions were monitored by thin layer chromatography on TLC silica gel 60 F254 plates for completion of the reaction; mobile phase solvents were selected as n-hexane: ethyl acetate (7:3). Melting points of all the synthesized compounds were checked in capillary tubes by using a melting point apparatus.

TABLE 3.1: List of chemicals

Sr. No	Name of chemical	Com.p...any/suppliers
1	Urea	Merck
2	Gaunidine	Sigma alderich
3	Benzene sulphonyl chloride	Chemco
4	Dimethyl sufoxide	Chemco
5	Methanol	Chemco
6	Ethyl accetate	Chemco
7	hexane	Chemco
8	aniline	Acros organics
9	p-Fluoro aniline	Acros organics
10	p-Chloro aniline	Acros organics
11	p-Nitro aniline	Acros organics
12	p-Bromo aniline	Acros organics
13	Benzoyl chlorode	Spectrochem
14	Benzoic acid	Merck
15	p-Nitro benzoic acid	Merck
16	p-Chloro benzoic acid	Merck
17	p-Fluoro benzoic acid	Merck
18	p-Methyl benzoic acid	Merck
19	Pyrroliodine	Merck
20	Piperazine 2 carboxylic acid	Sigma alderich
21	Thionyl chloride	Spectrochem
22	Chloro acetyl chloride	Chemco
23	Nitrobenzene	Chemco
24	Anhydrous $FeCl_3$	Merck
25	Tetrahydrofuran	Chemco

TABLE No 3.2: List of instruments used during research work

Sr No	Name of Instruments
1	UV chamber
2	FT-IR spectrometer (Bruker, Parul Institute of pharmacy, Vadodara)
3	NMR Spectrometer (Brüker Biospin, Switzerland) CoE Rajkot, Gujarat
4	Mass spectrophotometer (O2h discovery, Ahmedabad and Synzeal research Pvt Ltd, Gandhinagar)
5	Heating metal
6	Hot plate
7	Water bath
8	Magnetic stirrer
9	Glucometer (Johnson and Johnason)
10	Melting point apparatus

3.2 Experimental Section

3.2.1 Molecular Docking Study

3.2.1.1 Steps of ligand docking [44]

Docking is a method which forecasts the preferred positioning of one ligand when bound in an active site to form a continuous compound.

STEP 1 – Preparation of ligands

- Draw ligands using a Java applet and upload a single ligand file or multiple ligands.
- Draw molecule structures by MarvinSketch, which is Java based program by a continually growing list of editing structures and a quantity of patterns to make molecule drawing humbler.
- Upload a ligand in MDL MOL, SYBYL MOL2, PDB, SMILES format and multiple ligands in SDF format.
- Set up rotatable bonds and atom categories automatically or modify manually.
- Download the attached files in numerous file formats including mol, pdb, mol2 and pdbqt.
- Organize your ligands into self-defined folders.

STEP 2 – Preparation of proteins

- Upload protein structures from files or download them from the Protein Data Bank using Docking Server by providing the entry code or by text search.
- Select the protein chain, heteroatoms, ligands and waters present in the protein pdb file that you want to be perform in the docking calculation in the progression of protein setup.
- Set up the simulation box by one of the following ways:
- Select known binding site through a cocrystallized ligand.
- Select the center of mass of the protein molecules.
- Select the coordinates of the box center and amino acid residues that define the binding site
- Molecular Docking Server calculates essential map files for each atom form and prepares the input files for docking designs.

STEP 3 – Setup ligand protein docking calculations

- Select a protein and a ligand from your library.
- Modify advanced parameters during the simulation, such as number of runs, number of evaluations etc.

STEP 4 – Evaluation of results

- Choose an image from the image gallery or render in Molecular Docking Server.
- Analyze the secondary interactions between the protein and ligand.

3.2.1.2 Ligand Docking

The Crystal structure of the pancreatic ATP-sensitive K^+ channel SUR1/Kir6.2 complexed with ATP and glibenclamide (PDB ID: 5TWV) was imported. [45] Docking, screening and post-analysis of the designed molecules was completed using the iGEMDOCK program with the protein target 5TWZ. The binding sites of the symbols were organized and the energy minimized molecules were imported. During docking, the molecules were prepared and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands.

The docking, wizard ligand were selected and the scoring function used was iGEMDOCK score. If hydrogen bonding is possible, the hydrogen bond energy contribution to the docking score is allocated a penalty based on the deviations from the ideal bonding angle. This option can significantly reduce the number of unlikely hydrogen bonds and also internal electrostatic contact; internal hydrogen bond sp^2-sp^2 torsions are calculated from the pose by enabling the ligand evaluation relations.

The search algorithm is occupied as iGEMDOCK and numbers of runs taken are 70 and max interactions were 2000 with population size 200 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are verified and the one giving lowest energy is selected. If the energy is positive, then additional max positions will be tested. If the pose being docked is closer to one of the ligands in the list than quantified by the Root Mean Square Deviation (RMSD) threshold, an extra penalty term (the energy penalty) is added to the scoring function. This ensures a greater diversity of the returned solutions since the docking engine will focus its search on poses different from earlier poses found.

The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation standard docking were set. Docking was conducted between protein and inhibitor, which results in binding attractions in kcal/ mol. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was 8Å. The empirical scoring function of iGEMDOCK was estimated as:

Fitness = vdW + Hbond + Elec.

Here, the vdW term is vander Waal energy. H-bond and Elect terms are hydrogen bonding energy and electro-static energy, respectively.

3.2.1.3 In *sillico* toxicity study

Efforts to regulate the physicochemical assets that relate to long-term molecules feasibility has been conducted in concert with gathering biological statistics on attributes such as: cell toxicity, efflux liability, metabolic stability or inhibition, cell permeability, bioavailability, CNS permeability, protein binding, brain tissue binding, promiscuity, clearance and volumes of distribution. Armed with these results, dealings have been sought with a range of similar physicochemical belongings. Most of this care has been placed on easily calculated parameters such as molecular weight (MW), ClogP, polar surface area (PSA), the number of hydrogen bond donors/acceptors, aromatic character and, the number of rotatable bonds.

One of the first overall studies, to appear is the well-known work of Lipinski and coworkers who worried that problems are likely to be met with oral absorption if a compounds meets two or more of the criteria:

- Molecular weight > 500,
- C log P > 5,
- H-bond donors > 5
- H-bond acceptors > 10.

More recent work has been able to refine these strategies and this review will only highlight a subset of this research. [46]

3.2.2 Sci finder report [47]

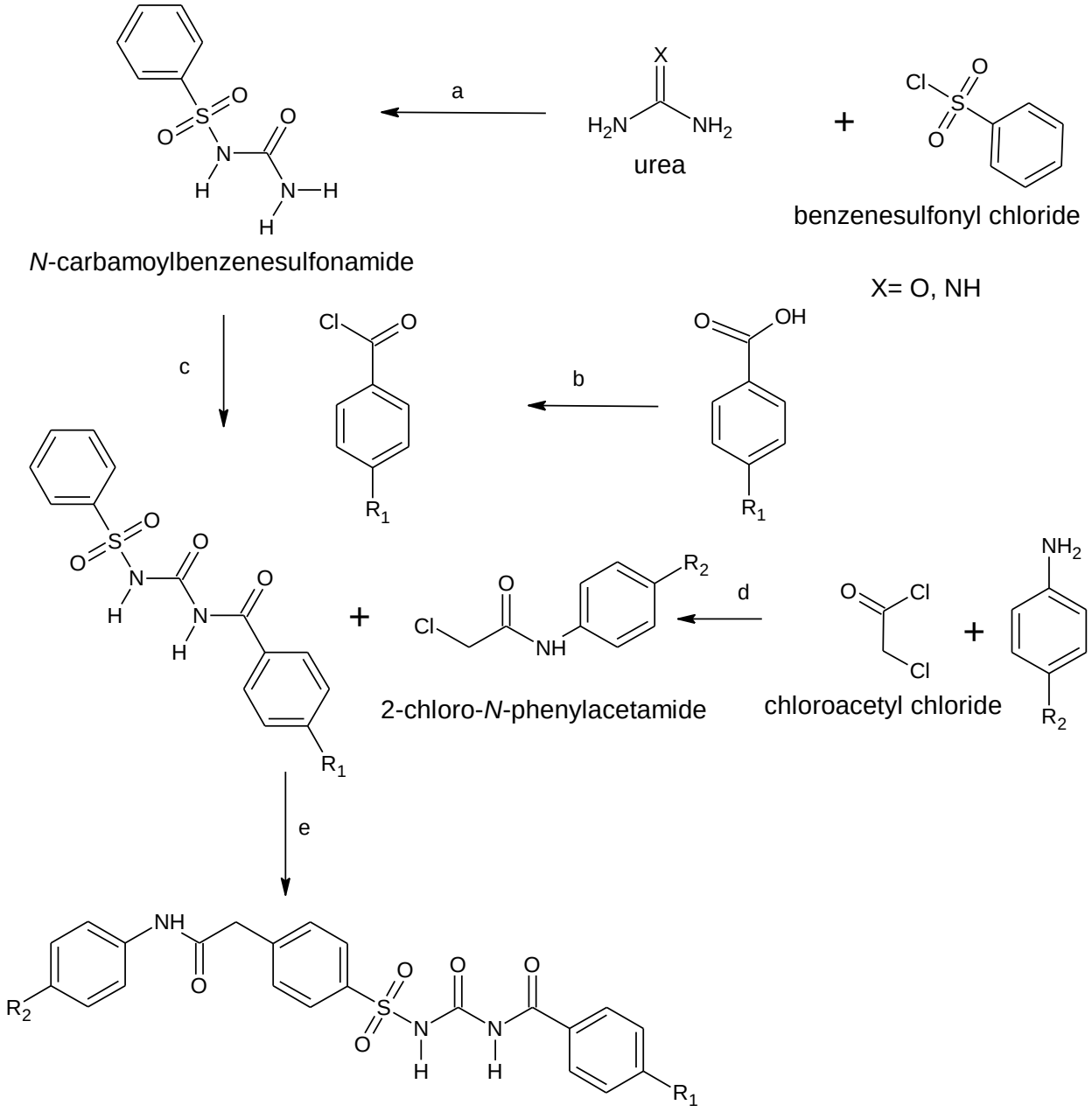
SciFinder was produced by Chemical Abstracts Service (CAS). Chemical literature, indexing journal articles and patent records, as well as chemical substances and reactions were searched by Sci finder.

Search option is available for topic, author and substances by name or CAS Registry Number, or use the editor to draw chemical structures, substructures or reactions.

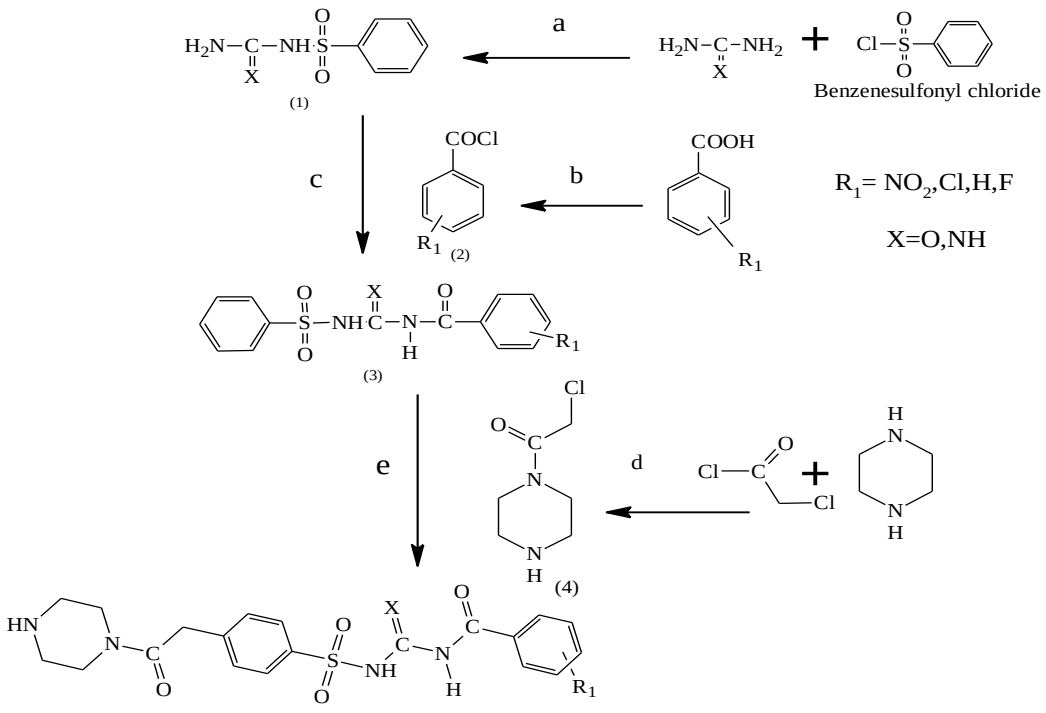
It is a core research tool for chemistry, biochemistry, chemical engineering, materials science, nanotechnology, physics, environmental science and other science and engineering field. Depending on your research, SciFinder is complementary to other databases like Reaxys, Web of Science, PubMed, INSPEC.

All the design compounds were checked by Sci finder software so its give clear idea about the novelty of compounds. Some images of Sci finder analysis are given in chapter 4.

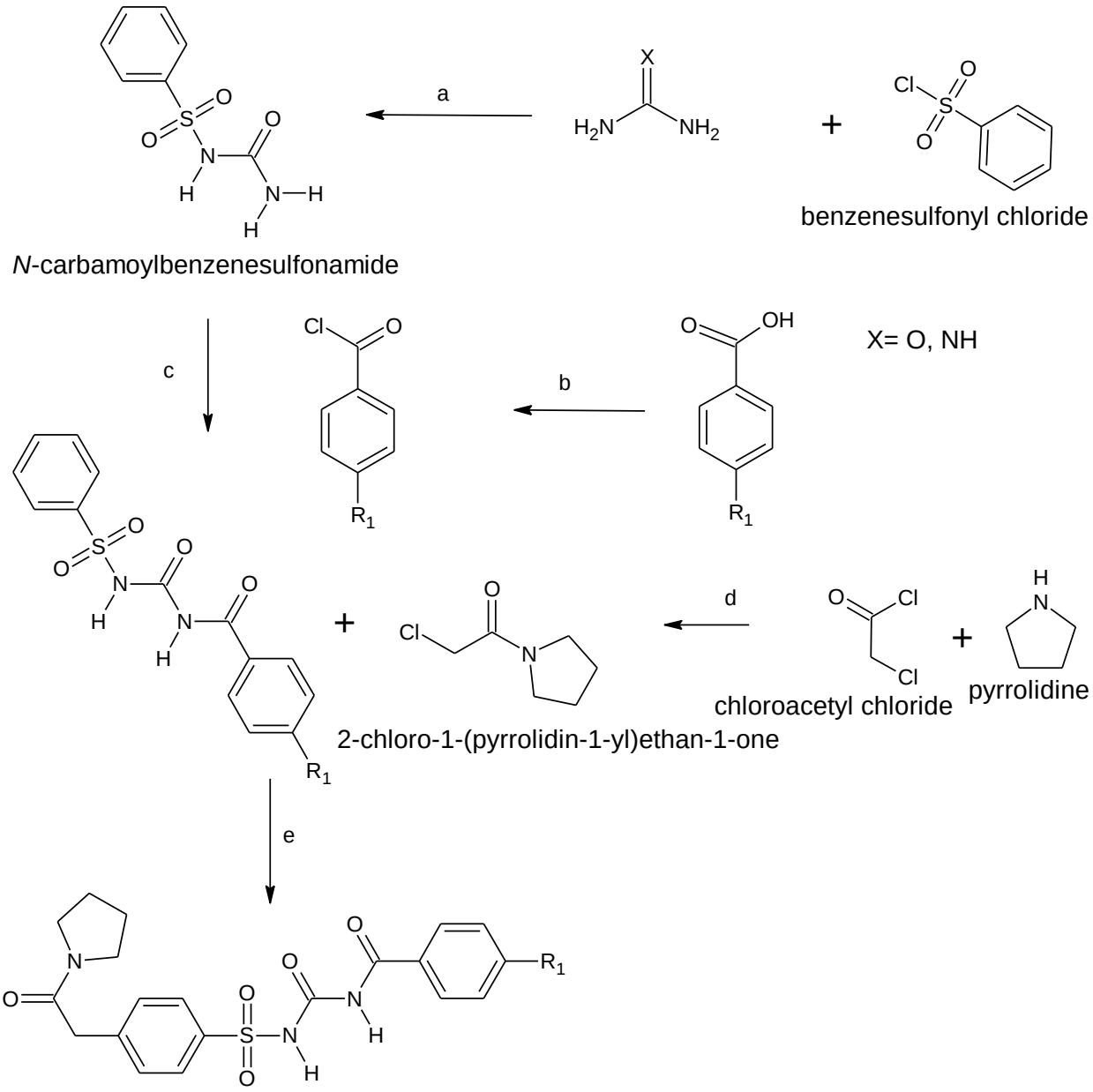
3.2.3 Scheme of synthesis



Scheme 1. Synthetic route for the preparation of the sulphonylureas/gaunidine derivatives Reagents and conditions: (a) TEA, CH_3CH_2OH, Reflux (yield >70%); (b) $SOCl_2$, reflux, 3 h, (yield >60%); (c) TEA, CH_3CH_2OH, Reflux (yield >80%); (d) DRY THF, 0 TO RT,Stirring ,4 h, rt (yield >85%). (e) Nitrobenzene, $FeCl_3$, reflux, 7 hrs



Scheme 2. Synthetic route for the preparation of the sulphonylureas/guanidine derivatives Reagents and conditions: (a) TEA, CH_3CH_2OH, Reflux (yield >70%); (b) $SOCl_2$, reflux, 3 h,(yield >60%); (c) TEA, CH_3CH_2OH, Reflux (yield >80%); (d) DRY THF, 0 TO RT, Stirring, 4 h (yield >85%). (e) Nitrobenzene, $FeCl_3$, reflux, 7 hrs



Scheme 3. Synthetic route for the preparation of the sulphonylureas/guanidine derivatives Reagents and conditions: (a) Pyridine, CH_3CH_2OH, Reflux (yield >75%); (b) $SOCl_2$, reflux, 3 h, (yield > 60%); (c) Pyridine, CH_3CH_2OH, Reflux (yield >70%); (d) Dry THF, 0°C to RT, Stirring, 4 h, RT (yield >85%). (e) Nitrobenzene, $FeCl_3$, reflux, 7 hrs

3.2.4 Procedure and Spectral characterizations

3.2.4.1 Synthesis of 1-Cyclohexanecarbonyl-3-(4-(2- (pyrazine-2-carboxamido) ethyl)phenylsulfonyl)guanidine (5a)

Reflux between N-(2-chloroethyl) pyrazine-2-carboxamide (0.1 mole) and 1-cyclohexanecarbonyl-3-(phenylsulfonyl) guanidine (1 mole) was done for 7 hrs in the presence of anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and liquid of 1-Cyclohexanecarbonyl-3-(phenylsulfonyl) guanidine was isolated. The Final product was obtained in a yield of 60% with b.p.: 100-102°C. R_f value: 0.4 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

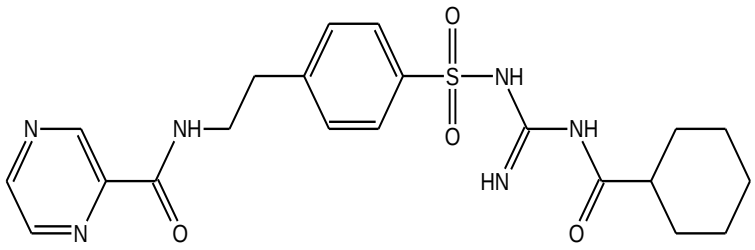


TABLE 3.3: Spectral data of 5a

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{13}C NMR (400 MHz)
5a	2977 (C-H) *str* Ar 1172 (S=O) *str* 2788 (C-H) *str* 2138(C=N) *str*	459 [M+1]	39.54 ($\underline{C}H_2CH_2Ar$), 38.28 ($CH_2\underline{C}H_2$,Ar), 141.12-145.05 (CH, pyrazine)

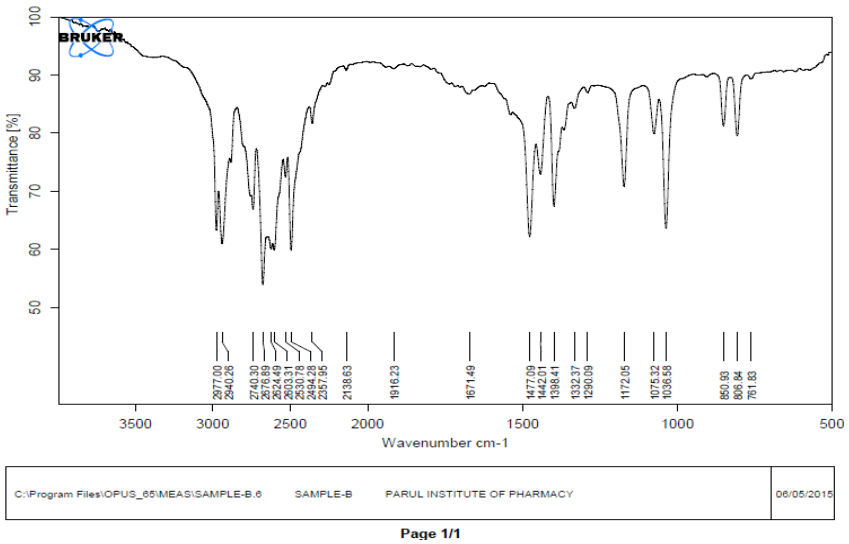


FIGURE 3.1: IR Spectra of 5a

3.2.4.2 Synthesis of 1-(4-(2-Benzamidoethyl) phenylsulfonyl)- 3-(cyclohexane carbonyl) urea (5b)

Reflux of N-(2-chloroethyl)benzamide (0.1 mole) and 1-cyclohexanecarbonyl-3-(phenylsulfonyl)urea (0.1 mole) was performed for 7 hrs in the presence of $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid white crystals were isolated with a yield of 75%. m.p.: 150-154°C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

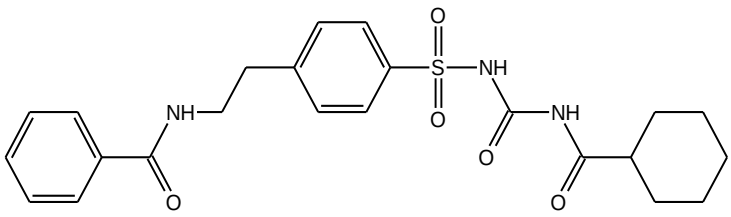


TABLE 3.4: Spectral data of 5b

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5b	1714 (C=O) *str* 2977 (C-H) *str* 1289 (S=O) *str*	454.6 [M-2]	2.31 (s, 2H, CH_2), 3.48 (s, 2H, CH_2), 1.32 (s, 11H, CH_2) Cyclohexane, 8.16 (s, 1H, NH), 8.16-8.33(m, 9H, Ar)

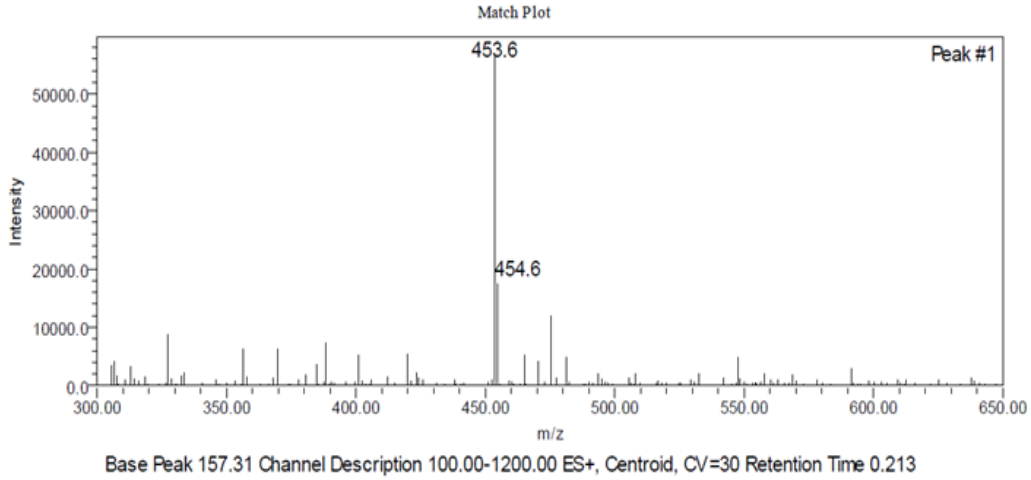


FIGURE 3.2: Mass Analysis report of 5b

3.2.4.31-(4-(2-(4-Fluorophenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)urea (5c)

Friedel–Crafts alkylation of 1-(4-nitrobenzoyl)-3-(phenylsulfonyl) urea (0.1 mole) and 2-chloro-N-(4-fluorophenyl)acetamide (1 mole) was finished for 7 hrs in the presence of anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and crystals were isolated. Yield: 65%; m.p.: 116-118°C. R_f value: 0.6 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

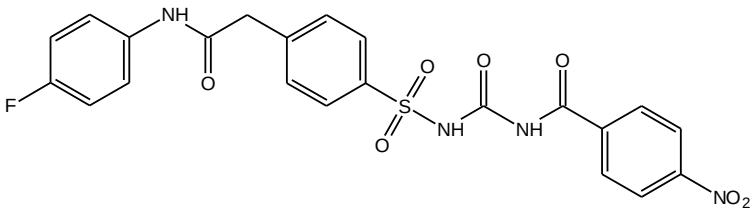


TABLE 3.5: Spectral data of 5c

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5c	3701 (N-H) *str* 1670 (C=O) *str* 2988 (C-H) *str* Ar, 1278 (S=O) *str* 2950 (C-H) *str* 1568 (N-O) *str* *1350* (N-O) *str*	500.5 [M]	^{1}H NMR (δ ppm): 3.45(s,2H,CH2),2.50(s,1H,NH),7.98(s,1H,NH),7.65(d,2H,ArH),7.16(d,2H,ArH),7.60(d,6H,ArH),8.20(d,2H,ArH);

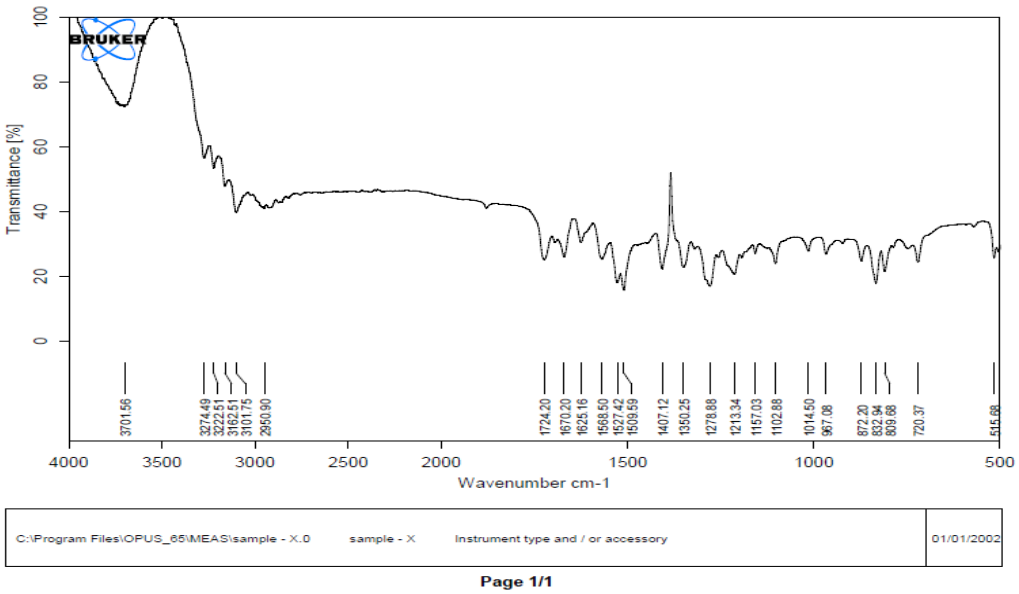


FIGURE 3.3: IR spectra of 5c

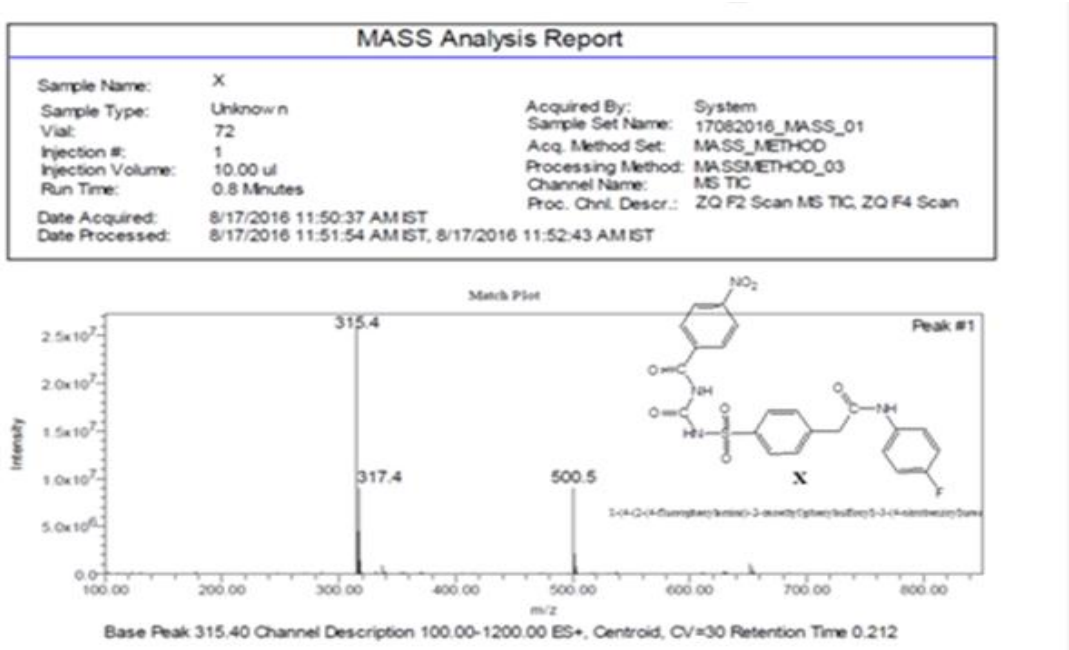


FIGURE 3.4: Mass Analysis report of 5c

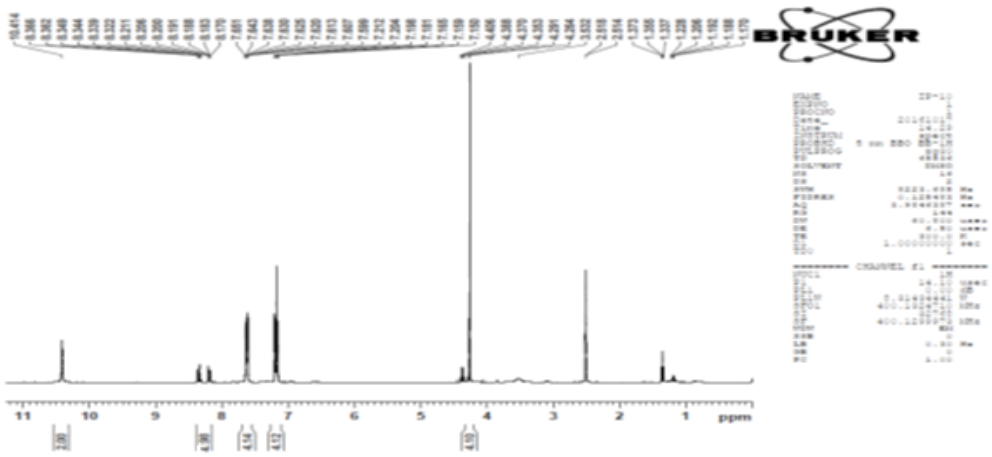


FIGURE 3.5: ^{1}H NMR of 5c

3.2.4.4 1-Benzoyl-3-(4-(2-(3-chlorophenylamino)-2-oxoethyl) phenylsulfonyl) urea(5d)

Reflux of 2-Chloro-N-(3-chlorophenyl) acetamide (1 mole) and 1-benzoyl-3-(phenylsulfonyl) urea (1 mole) was performed for 7 hrs in the presence anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid white crystals were isolated with a yield of 60%. m.p.: 112-114 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

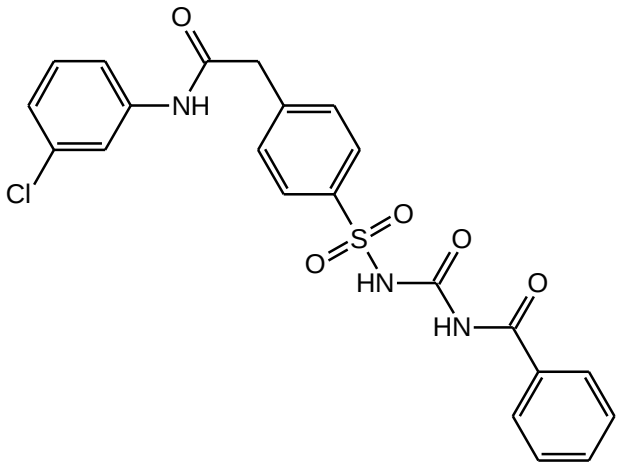


TABLE 3.6: Spectral data of 5d

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5d	3568 (N-H) *str* 1671 (C=O) *str* 2999(C-H) *str* Ar, 1295 (S=O) *str* 822 (C-Cl) *str*	474 [M+1]	2.31 (s, 2H, CH_2), 3.48 (s, 2H, CH_2), 10.41(s,1H,NH), 8.32(s, 1H,NH),8.17(s,1H,NH) , 7.85- 7.90(d,4H,ArH), 7.65-7.85(d,4H,ArH), 7.50(s, 1H,ArH),7.52(s,1H, ArH)

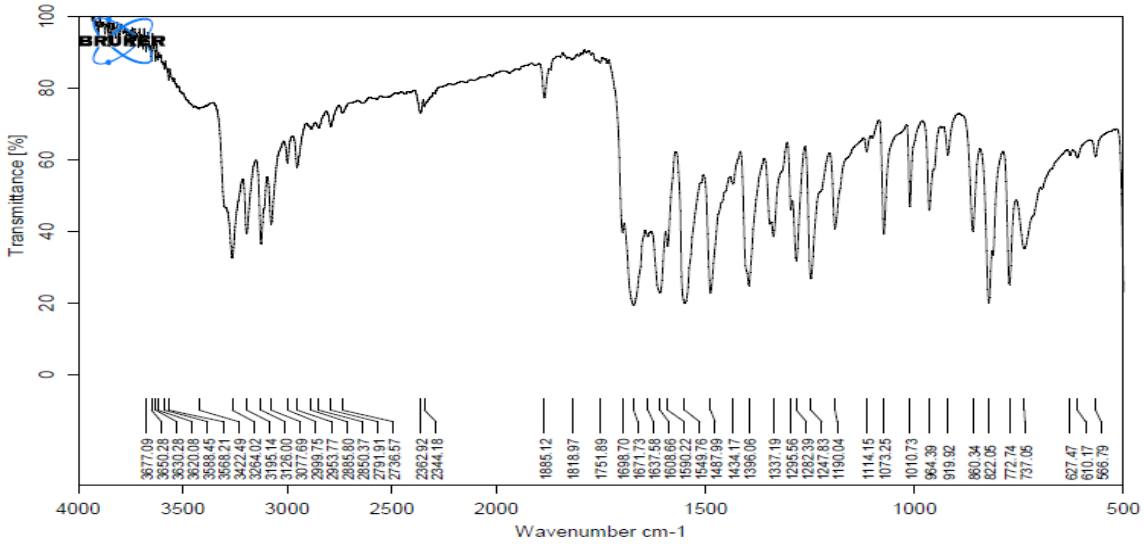


FIGURE 3.6: IR spectra of 5d

3.2.4.5 1-(4-(2-Benzamidoethyl)phenylsulfonyl)-3-cinnamoyl guanidine (5e)

Reaction between 1-cinnamoyl-3-(phenylsulfonyl) guanidine (0.1mole) and N-(chloromethyl)benzamide (0.1mole) was performed for 6 hrs in the presence anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid white crystals were isolated with a yield of 70%. m.p.: 118-120 °C. R_f value: 0.7 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

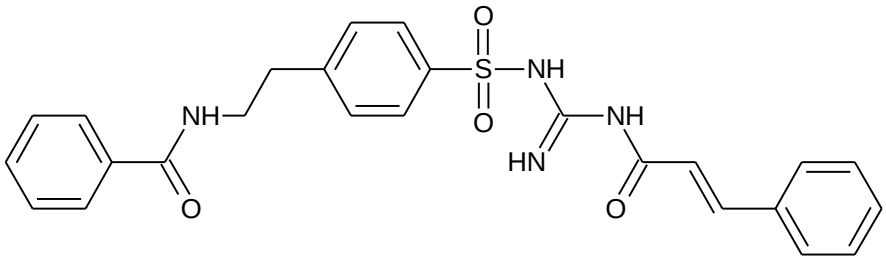


TABLE 3.7: Spectral data of 5e

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5e	3417 (N-H) *str* 1720(C=O) *str* 2988 (C-H) *str* Ar, 1276 (S=O) *str* 1602 (C=C) str	476 [M+1]	1.2-1.29(t,4H,CH2),4.28(s,3H, NH) 7.96(s,2H,NH),7.950-7.935(d,4H,ArH),7.60-7.61(d,2H,ArH),7.47-7.48(d,4H,ArH), 7.49(s,1H,ArH)

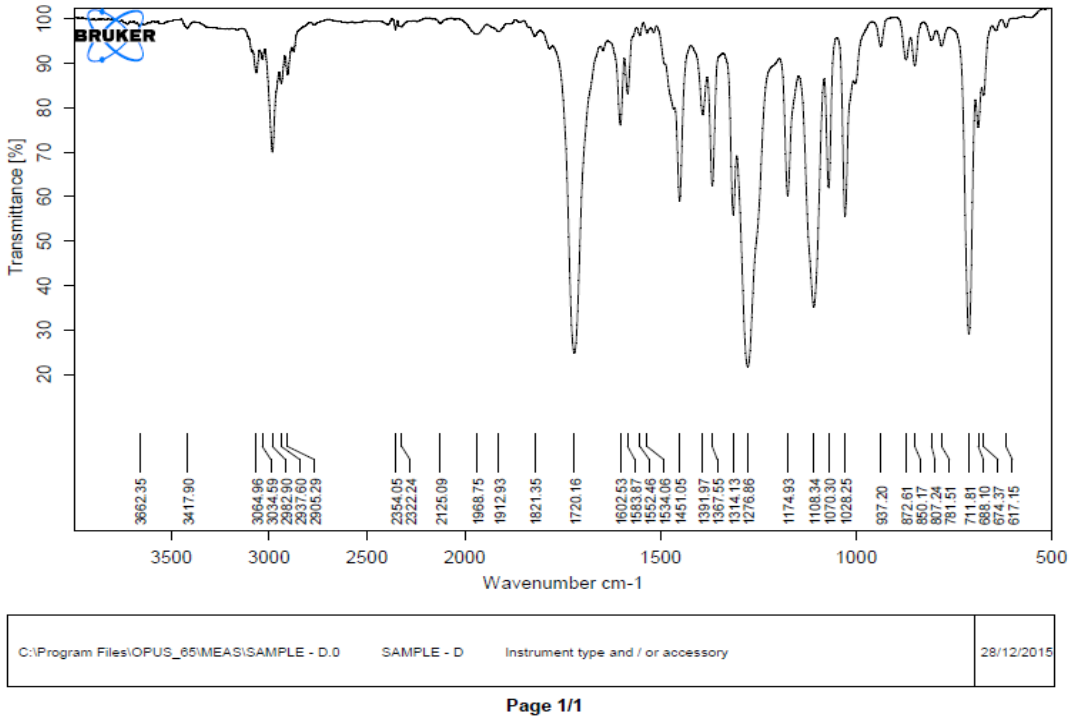


FIGURE 3.7: IR spectra of 5e

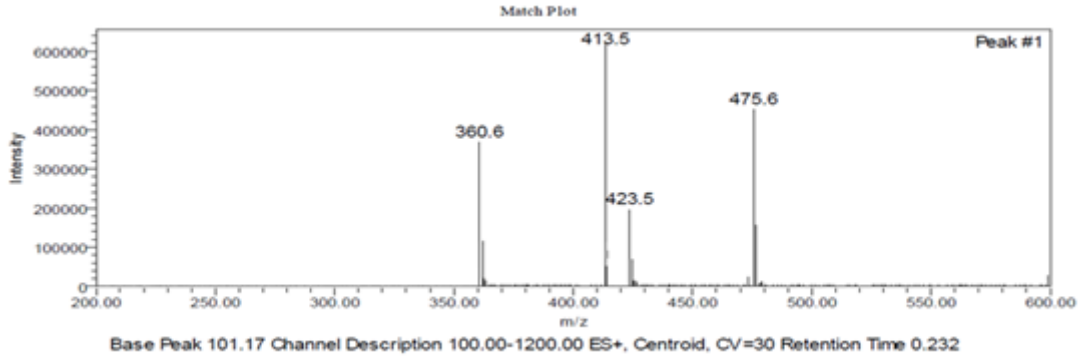


FIGURE 3.8: Mass analysis of 5e

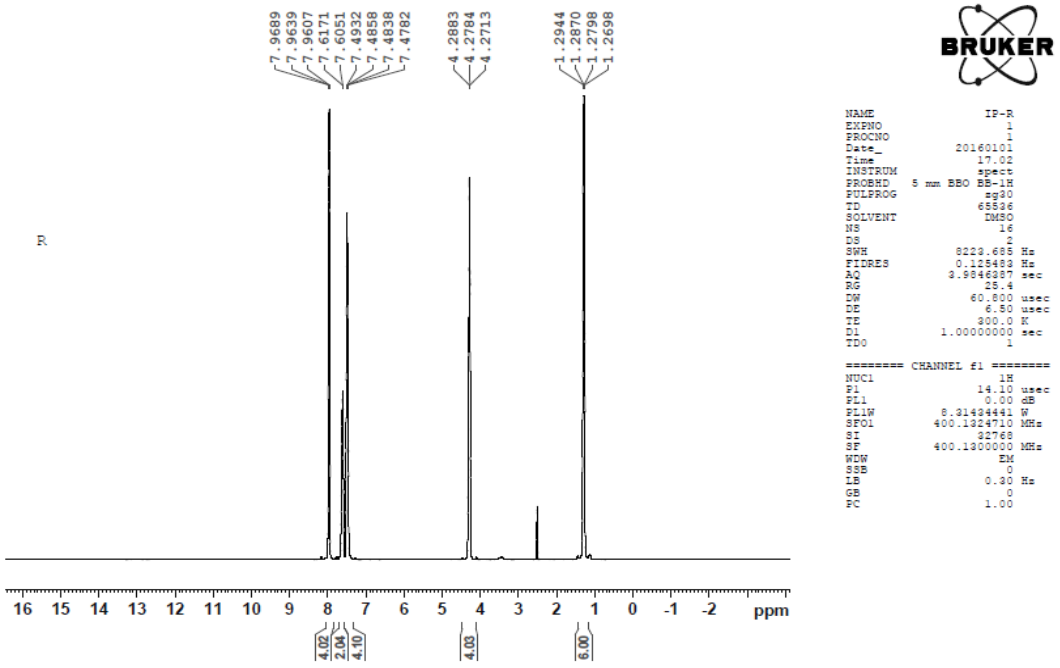


FIGURE 3.9: ^{1}H NMR of 5e

3.2.4.6 1-(2-Chlorobenzoyl)-3-(4-(2-(4-fluorophenylamino)-2-oxoethyl)phenylsulfonyl)urea (5f)

Friedel–Crafts alkylation of 1-(2-chlorobenzoyl)-3-(phenylsulfonyl) urea(1 mole) and 2-chloro-N-(4-fluorophenyl) acetamide (1 mole) was done for 7 hrs in the presence of anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid white crystals were isolated with a yield of 52%, m.p.: 78°C. R_f value: 0.6 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

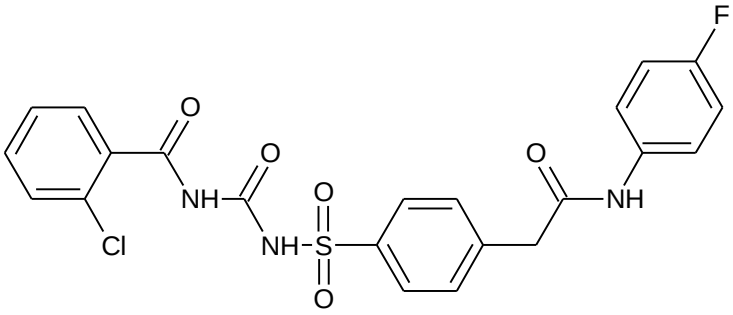


TABLE No 3.8: Spectral data of 5f

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5f	1669 (C=O) *str* 1247 (S=O) *str* 1113 (C-F) *str* 1508 (C-H) 2994 (C-H) *str* Ar, 3452 (N-H) *str*	488 [M-1]	3.45(s,2H,CH2),2.50(s,1H,NH),7.98(s,1H,NH),7.65(d,2H ,ArH),7.16(d,2H,ArH),7.60(d,6H,ArH),8.20(d,2H,ArH).

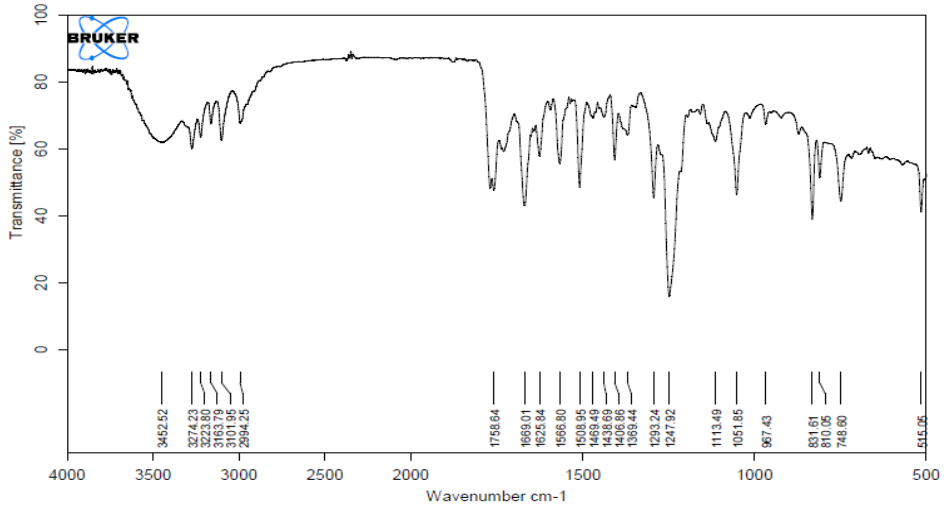


FIGURE 3.10: IR Spectra of 5f

3.2.4.7 1-(2-Chlorobenzoyl)-3-(4-(2-(4-fluorophenylamino)-2 oxoethyl)phenylsulfonyl)guanidine (5g)

Friedel–Crafts alkylation of 1-(2-chlorobenzoyl)-3-(phenylsulfonyl)guanidine(1 mole) and 2-chloro-N-(4-fluorophenyl)acetamide (1 mole) was done for 7 hrs in the presence of $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid gray crystals were isolated with a yield of 47%. m.p.: 98 °C. R_f value: 0.4 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

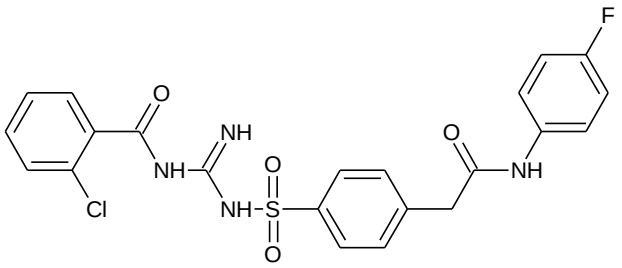


TABLE 3.9: Spectral data of 5g

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)	^{13}C NMR
5g	1738(C=O)*str* 1246(S=O) *str* 1050(C-F) *str* 1469(C-H) 2985(C-H) *str* Ar 3449(N-H) *str*	487 [M+1]	3.45(s,2H,CH2),2.50(s,1H,NH), 7.98(s,1H,NH),7.65(d,2H,ArH), 7.16(d,2H,ArH),7.60(d,6H,ArH) ,8.20(d,2H,ArH)	157.04(s,CF),38.81-40.07(t,CH2-Ar),164.53(s, CONH),115.31-134.88(m, CH-Ar)

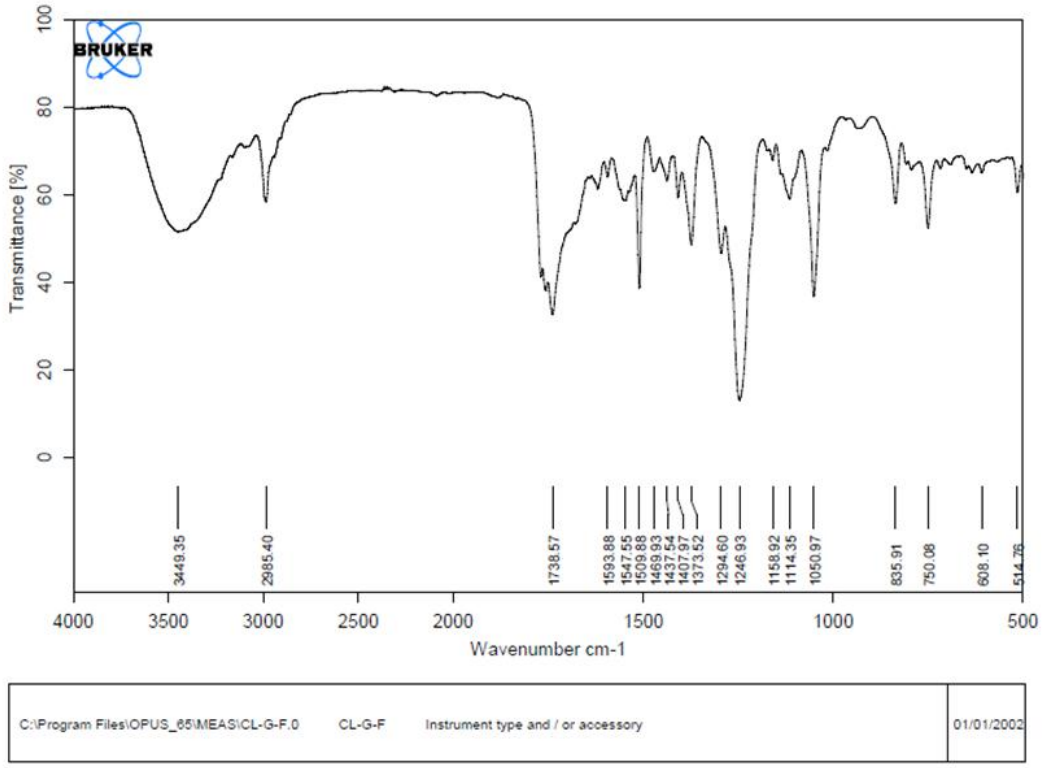


FIGURE 3.11: IR spectra of 5g

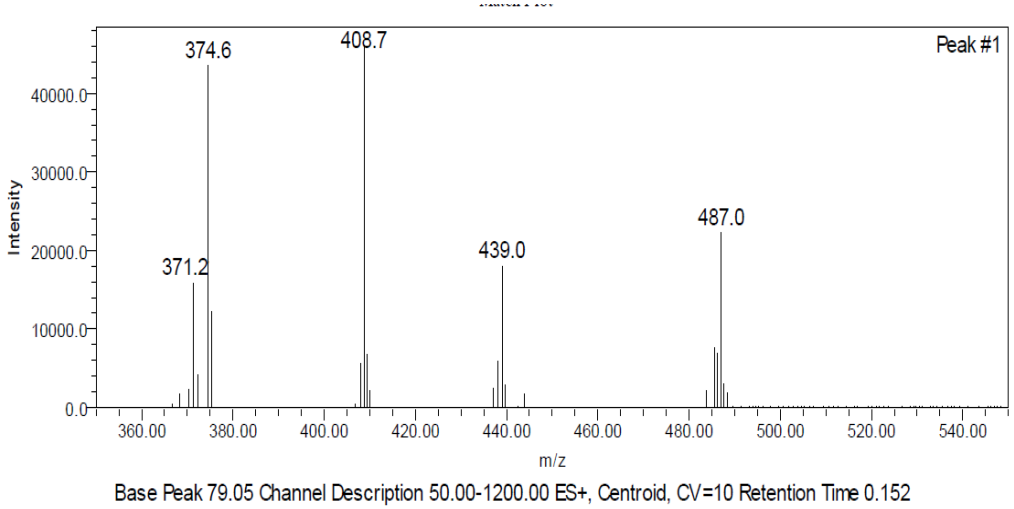


FIGURE 3.12: Mass analysis of 5g

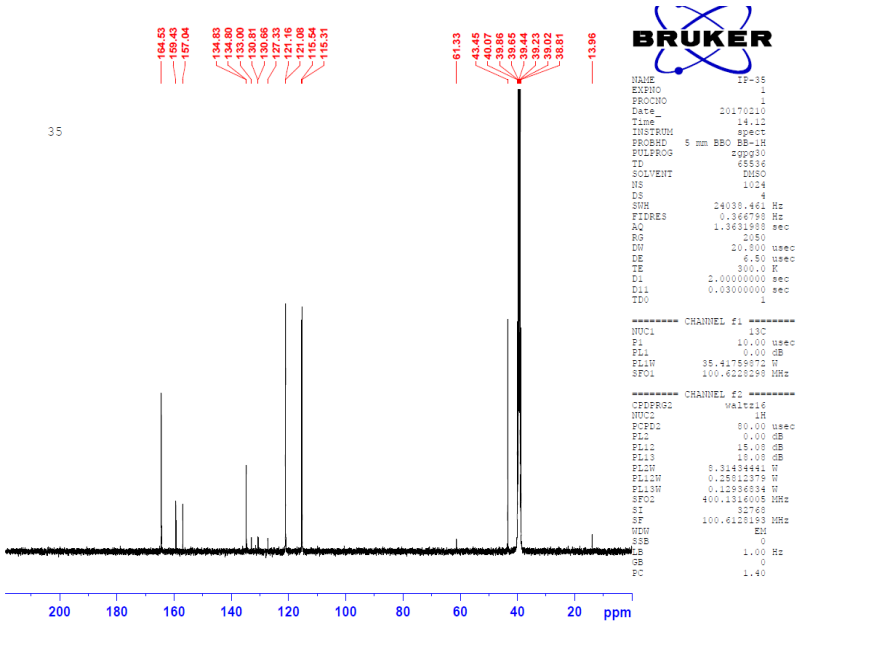


FIGURE 3.13: ^{13}C NMR of 5g

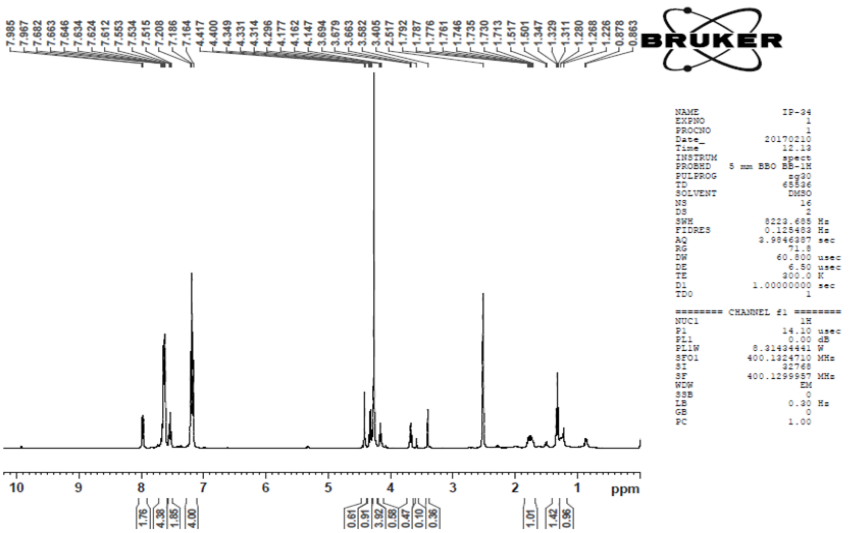
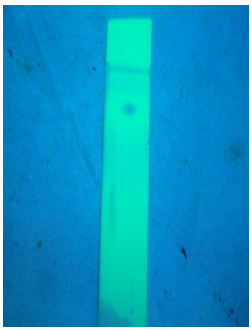


FIGURE 3.14: ^{1}H NMR of 5g

3.2.4.8 1-(Benzoyl)-3-(4-(2-(4-fluorophenylamino)-2-oxoethyl)phenylsulfonyl)guanidine (5h)

1-Benzoyl-3-(phenylsulfonyl) guanidine (1 mole) and 2-Chloro-N-(4-fluorophenyl) acetamide (1 mole) were reacted in 250 ml round bottom flask for 7 hrs in the presence of $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and gray crystals were isolated with a yield of 72%. m.p.: 140-142 °C. R_f value: 0.7 (mobile phase: ethyl acetate: hexane: 0.7:0.3)



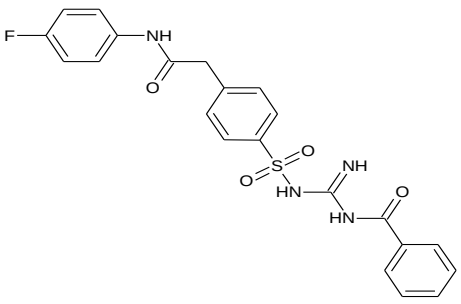


TABLE 3.10: Spectral data of 5h

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5h	1717(C=O)*str*, 1175(S=O) *str*, 1314 (C-F) 1451 (C-H) 2983 (C-H) *str* 3467(N-H) *str*	453.2 [M+1]	3.40-3.69(t, 4H, CH_2) 2.51(s,1H, NH),7.98(s,2H,NH),7.33-7.46(d,6H, ArH),7.80-7.89(d,4H,ArH), 7.12(d,2H,ArH), 7.44(s, 1H,ArH)

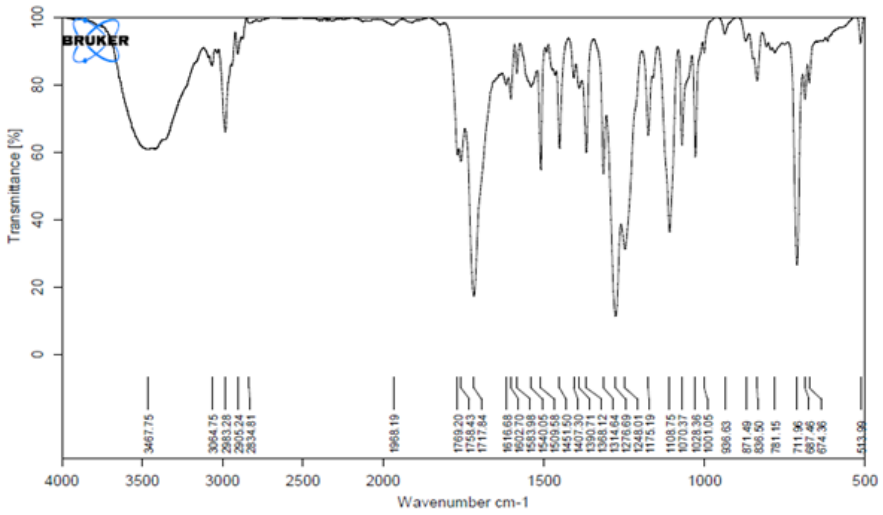


FIGURE 3.15: IR spectra of 5h

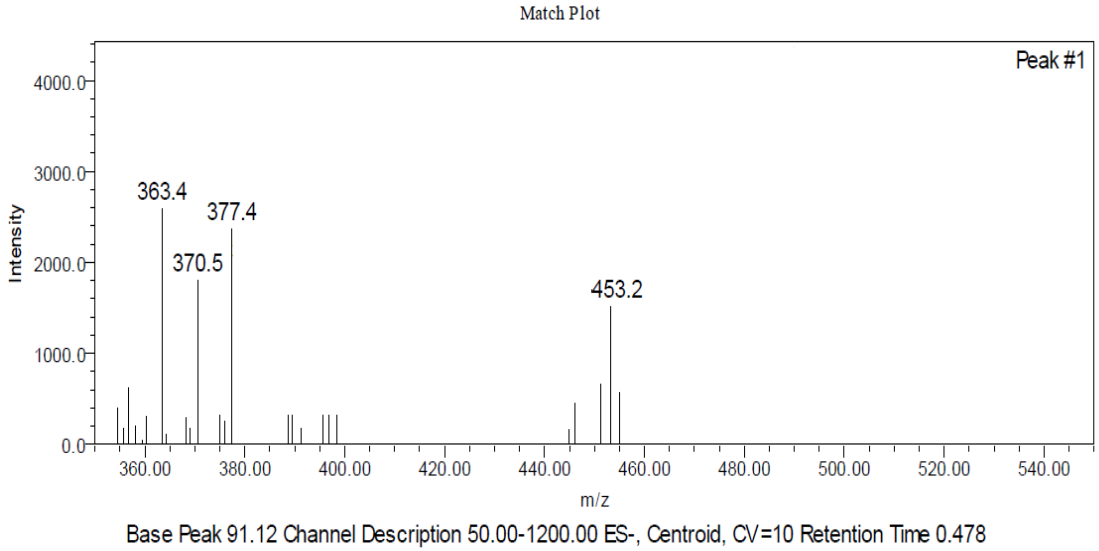


FIGURE 3.16: Mass analysis of 5h

3.2.4.9 1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(phenylamino) ethyl) phenylsulfonyl)urea (5i)

1-(4-Nitrobenzoyl)-3-(phenylsulfonyl) urea (1 mole) and 2-Chloro-N-phenylacetamide (1 mole) were reacted for 6 hrs in the presence of $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid light yellow crystals were isolated with a yield of 72%. m.p.: 102-104°C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

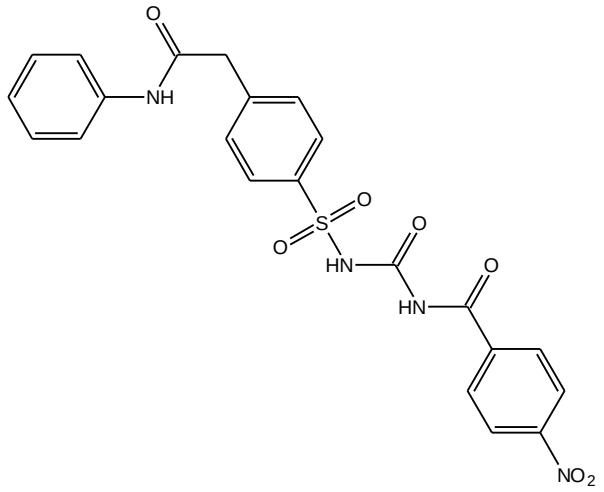


TABLE 3.11: Spectral data of 5i

Code	IR(KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5i	3416 (N-H) *str* 1716 (C=O) *str* 1684 (C-H) 3095 (C-H) 1170 (S=O) 1524 (NO) *str* 1350 (NO) *str*	480 [M-2]	3.40-3.69(t,4H,CH2),2.51(s,1H, NH),7.98(s,2H,NH),7.46-7.80(d,6H, ArH), 8.15-8.26(d,4H,ArH), 7.22(d,2H,ArH), 7.0(s,1H,ArH)

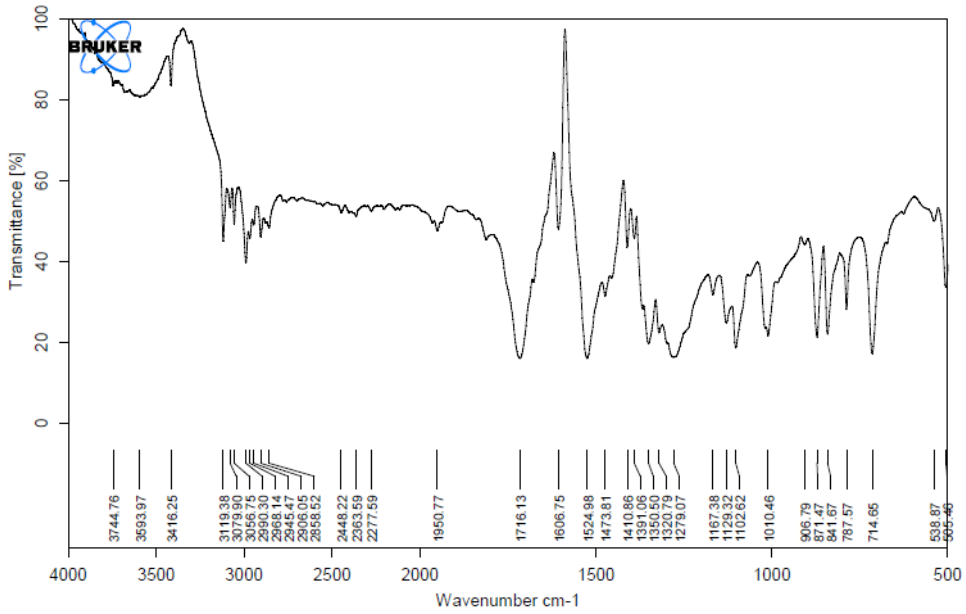


FIGURE 3.17: IR spectra of 5i

3.2.4.10 1-Cyclohexanecarbonyl-3-(4-(3-(4-nitrophenyl)-1-carboxamido ethyl)phenylsulfonyl)guanidine (5j)

1-Cyclohexanecarbonyl-3-(phenylsulfonyl) guanidine (1 mole) and N-(2-chloroethyl)-4-nitrobenzamide (1 mole) were reacted for 6 hrs in the presence of anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid crystals were isolated with a yield of 70%. m.p.: 140-142 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

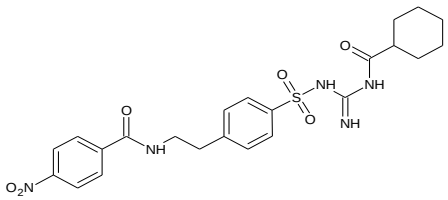


TABLE 3.12: Spectral data of 5j

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5j	3417 (N-H) *str*, 1726 (C=O) *str* 1608 (C-H) 3082 (C-H) *str Ar*, 1529 (NO) *str* 2857 (C-H) 1172(S=O)	503.6 [M-1]	3.40-3.69(t,4H,CH_2),2.51(s,1H, NH),7.98(s,2H,NH),7.46-7.90(d,4H, ArH), 8.15-8.26(d,4H,ArH)

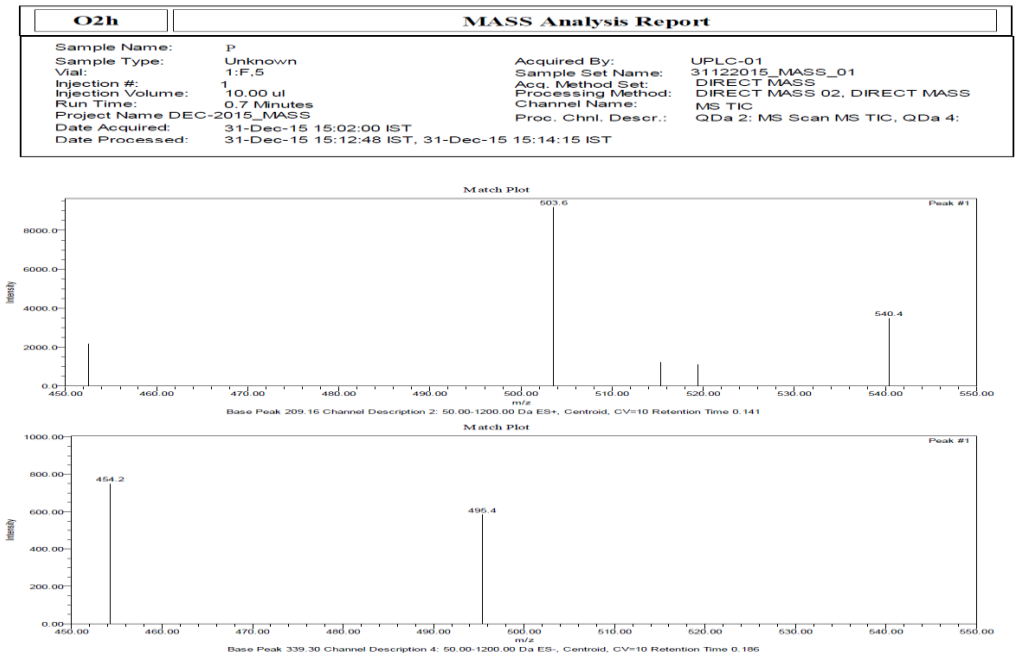


FIGURE 3.18: Mass analysis of 5j

3.2.4.11 1-benzoyl-3-(4-(2-oxo-2-(piperazin-1-yl)ethyl)phenylsulfonyl)urea (5k)

2-Chloro-1-(piperazin-1-yl)ethanone (0.1 mole) and 1-Benzoyl-3-(phenylsulfonyl)urea (0.1 mole) were reacted for 7 hrs in the presence of anhydrous $FeCl_3$ and Nitrobenzene as solvent. Reaction mixture was cooled and solid crystals were isolated with a yield of 60% m.p.: 140-142 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

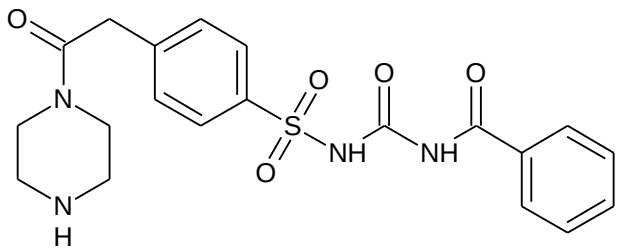


TABLE 3.13: Spectral data of 5k

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5k	1719 (C=O) *str* 1510 (C-H) 2927 (C-H) 1275 (S=O) *str*	431.6 [M+1]	8.022-8.053(d,4H,ArH), 7.35(d,4H,ArH),1.99(s,2H,NH), 1.11-1.22(t,4H,Piperazine), 1.31-1.77(t,4H,Piperazine)

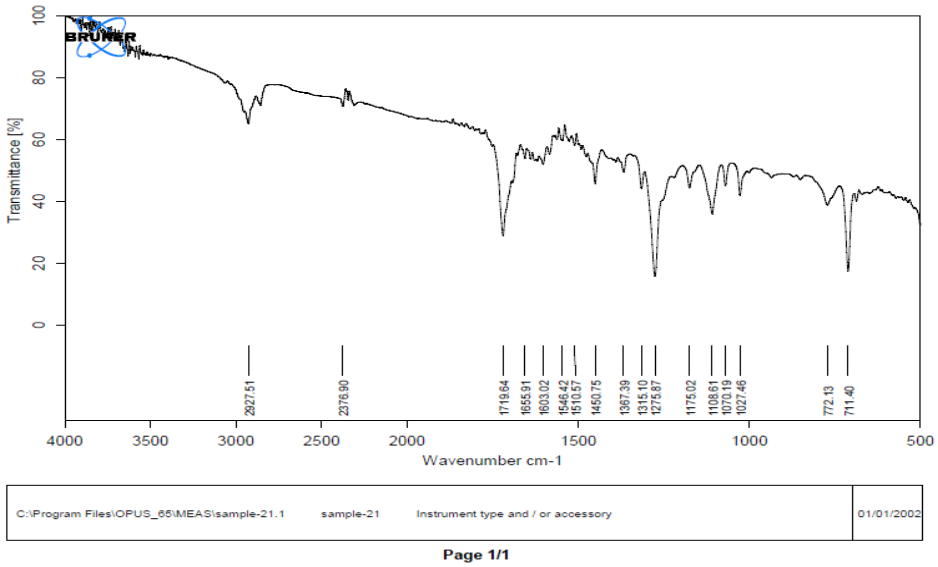


FIGURE 3.19: IR spectra of 5k

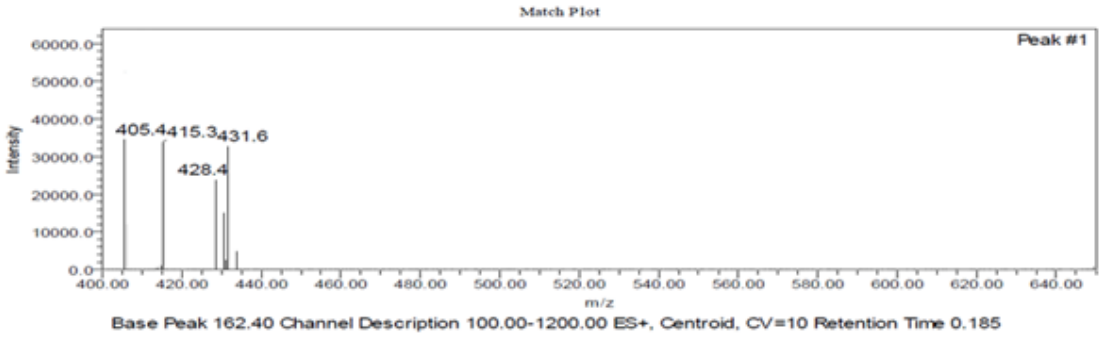


FIGURE 3.20: Mass analysis of 5k

3.2.4.12 1-(4-fluorobenzoyl)-3-(4-(2-oxo-2-(piperazin-1-yl)ethyl)phenylsulfonyl)urea(5l)

Friedel–Crafts alkylation of 1-(4-fluorobenzoyl)-3-(phenylsulfonyl) urea (0.1 mole) and 2-chloro-1-(piperazin-1-yl)ethanone(0.1 mole) in the presence of $FeCl_3$ and Nitrobenzene. Yield 45%, m.p.: 110-114 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

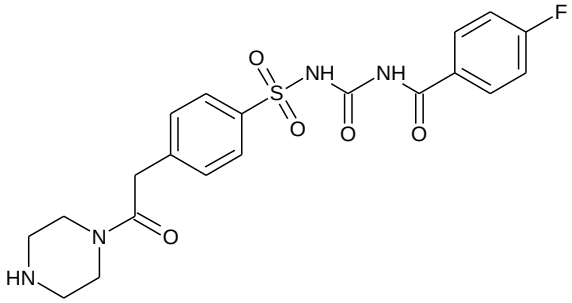


TABLE 3.14: Spectral data of 5l

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5l	1719 (C=O) *str* 1510 (C-H) 2927 (C-H) 1275 (S=O) *str* 1367 (C-F) str	450.1 [M+1]	8.022-8.053(d,4H,ArH), 7.35(d,4H,ArH),1.99(s,2H,NH), 1.11-1.22(t,4H,Piperazine), 1.31-1.77(t,4H,Piperazine)

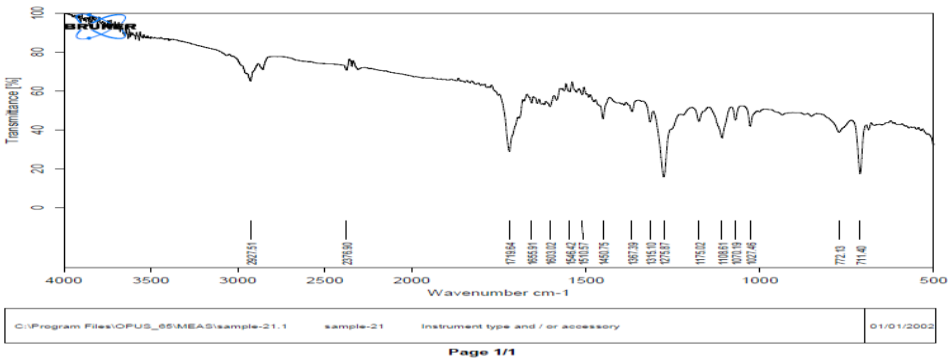


FIGURE 3.21: IR spectra of 5l

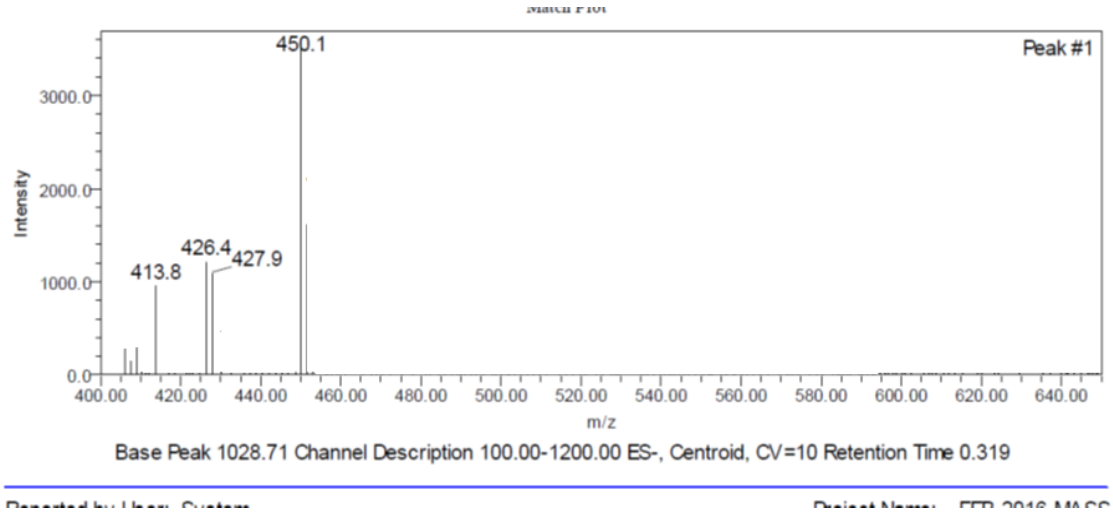


FIGURE 3.22: Mass analysis of 5l

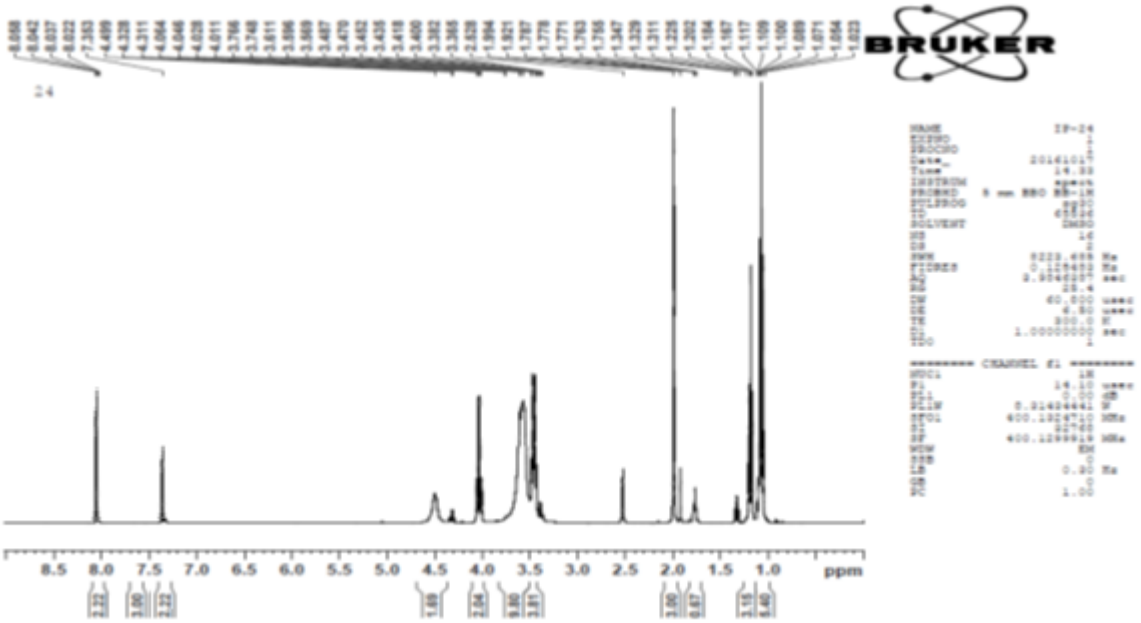


FIGURE 3.23:^{1}H NMR of 5l

3.2.4.13 1-(4-(2-(4-chlorophenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-fluorobenzoyl) guanidine (5m)

Friedel–Crafts alkylation of 1-(4-fluorobenzoyl)-3-(phenylsulfonyl) guanidine (0.1 mole) and 2-chloro-N-(4-chlorophenyl) acetamide (0.1 mole) in the presence of $FeCl_3$ and Nitrobenzene. Yield 50%, m.p.: 122 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

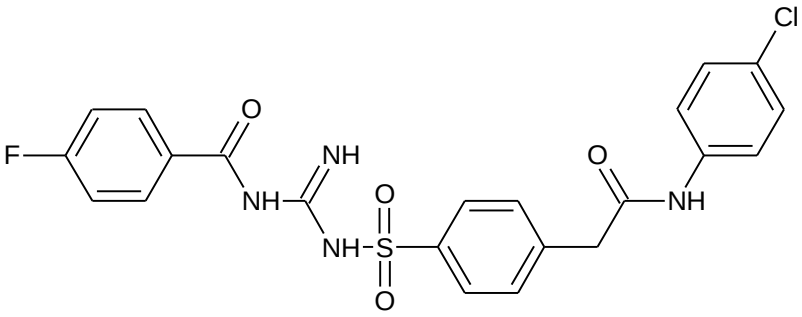


TABLE 3.15: Spectral data of 5m

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)	^{13}C NMR
5m	3500(N-H) *str*, 1700(C=O) *str*, 1500(C-H), 1157 (S=O) *str*	488 [M-1]	3.40-3.69(t,4H,CH_2),2.51(s,1H, NH),7.98(s,2H,NH),7.16-7.17(d,2H, ArH),7.20-7.53(d,4H,ArH),7.54-7.64(d,6H,ArH), 7.66(s,1H,ArH), 7.31(s,1H,ArH)	157.04(s, C-F),38.81-40.07(t,CH2-Ar),164(s, CONH),115.31-134.88(m, CH-Ar)

3.2.4.14 1-(2-chlorobenzoyl)-3-(4-(2-(4-fluorophenylamino)-2-oxoethyl)phenylsulfonyl)urea (5n)

Friedel–Crafts alkylation of 2-chloro-N-(4-fluorophenyl) acetamide (0.1 mole) and, 1-(2-chlorobenzoyl) -3-(phenylsulfonyl) urea (0.1 mole) in the presence of $FeCl_3$ and Nitrobenzene. Yield 60%, m.p.: 124 °C. R_f value: 0.6 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

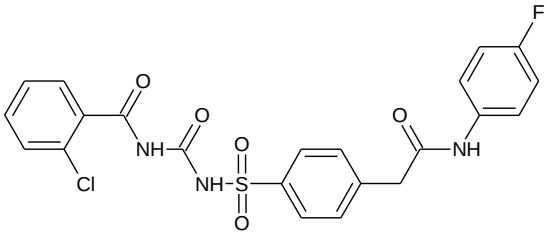


TABLE 3.16: Spectral data of 5n

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)	^{13}C NMR
5n	3500 (N-H) *str*, 1700 (C=O) *str*, 1500 (C-H), 1157 (S=O) *str*	488 [M-1]	3.40-3.69(t,4H,CH_2),2.51(s,1H, NH),7.98(s,2H,NH),7.16-7.17(d,2H, ArH),7.20-7.53(d,4H,ArH),7.54-7.64(d,6H,ArH), 7.66(s,1H,ArH), 7.31(s,1H,ArH)	157.04(s, C-F),38.81-40.07(t,CH2-Ar),164(s, CONH),115.31-134.88(m, CH-Ar)

3.2.4.15 1-(2-chlorobenzoyl)-3-(4-(2-(4-fluorophenylamino)-2 oxoethyl)phenylsulfonyl)guanidine (5o)

Friedel–Crafts alkylation of 1-(2-chlorobenzoyl)-3-(phenylsulfonyl) guanidine (0.1 mole) and 2-chloro-N-(4-fluorophenyl) acetamide (0.1 mole) in the presence of $FeCl_3$ and Nitrobenzene. Yield 65%, m.p.: 104-106 °C. R_f value: 0.6 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

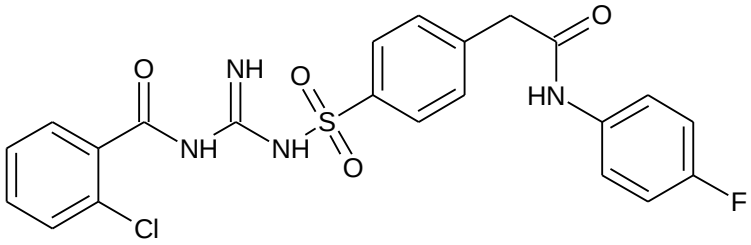


TABLE 3.17: Spectral data of 5o

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)	^{13}C NMR
5o	1738 (C=O) *str*, 1158 (S=O) *str* 750 (C-Cl) *str* 1509 (C-H) 2985(C-H) 3449(N-H) *str* 1129(C-F) *str*	487.6 [M]	3.40-3.69(t,4H,CH_2),2.51(s,1H, NH),7.98(s,2H,NH),7.16-7.17(d,2H, ArH),7.20-7.53(d,4H,ArH),7.54-7.64(d,6H,ArH), 7.66(s,1H,ArH), 7.31(s,1H,ArH);	157.04 (s, C-F), 38.81-40.07(t,CH2-Ar), 164(s,CONH), 115.31-134.88(m, CH-Ar)

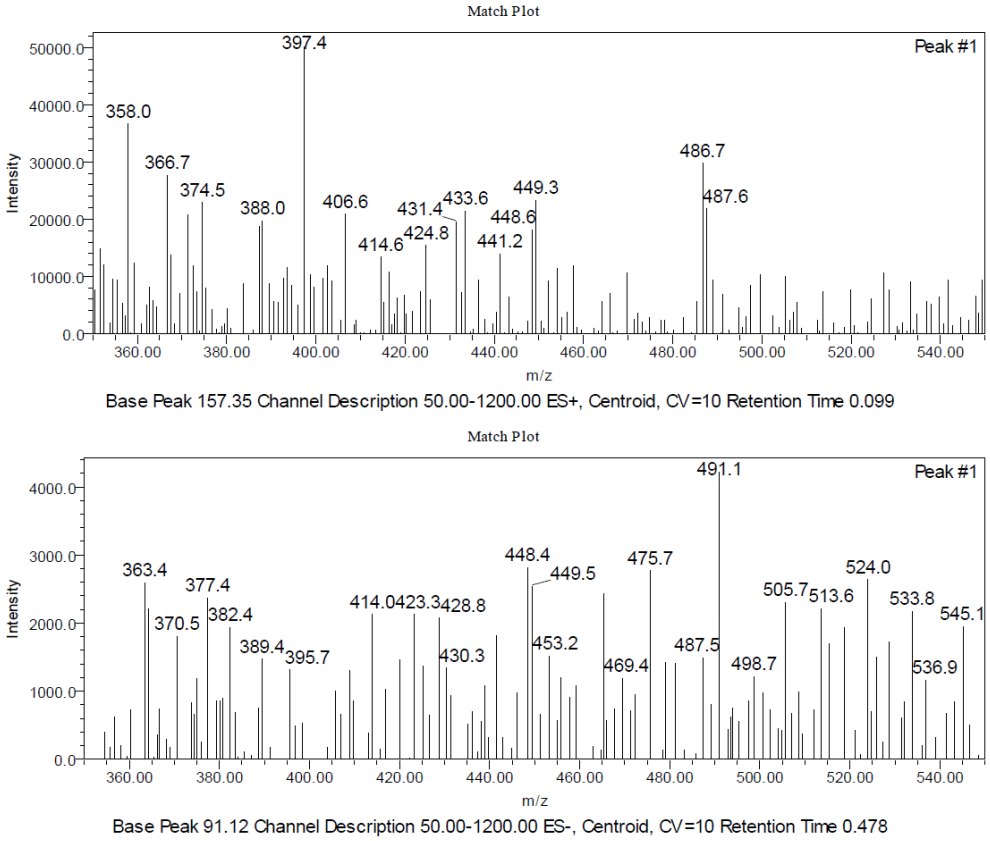


FIGURE 3.24: Mass spectra of 5o

3.2.4.16 1-(4-(2-(4-chlorophenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)urea (5p)

Friedel–Crafts alkylation of 1-(4-nitrobenzoyl)-3-(phenylsulfonyl) urea (0.1 mole) and 2-chloro-N-(4-chlorophenyl) acetamide were done in the presence of $FeCl_3$ and Nitrobenzene. Yield 60%, m.p.: 132-134 °C. R_f value: 0.5 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

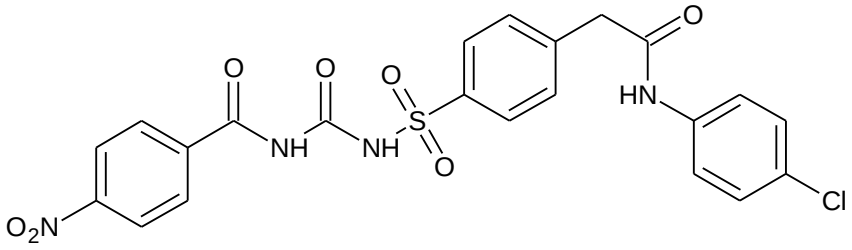


TABLE 3.18: Spectral data of 5p

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)	^{13}C NMR
5p	3452(N-H)*str* 1669(C=O) *str* 1684(C-H) 3101(C-H) 1247(S=O) *str* 1525(N-O) *str* 1369(N-O) *str*	516.2 [M] 514.5 [M-1]	^{1}H NMR: 3.40-3.69(t,4H,CH_2),2.51(s,1H, NH),7.98(s,2H,NH),7.16-7.17(d,2H, ArH),7.20-7.53(d,4H,ArH),7.54-7.64(d,6H,ArH), 7.66(s,1H,ArH), 7.31(s,1H,ArH)	157.04(s, C-F),38.81-40.07(t,CH2-Ar),164(s, CONH),115.31-134.88(m, CH-Ar).

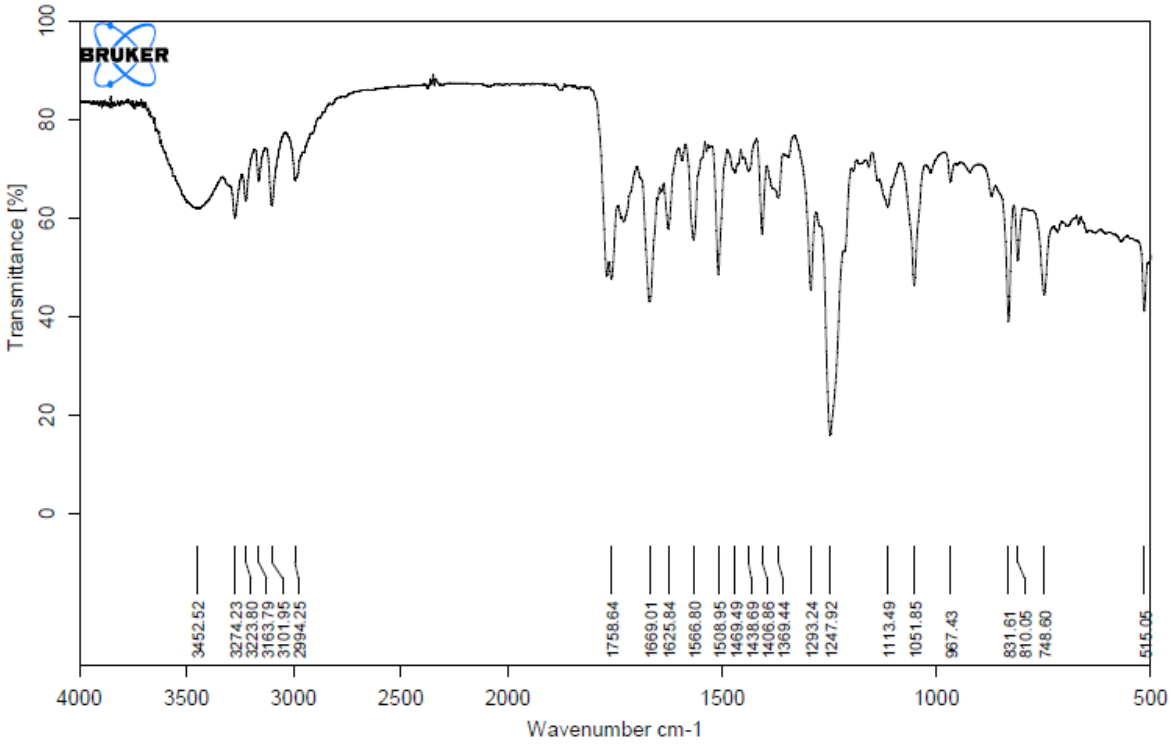


FIGURE 3.25: IR spectra of 5p

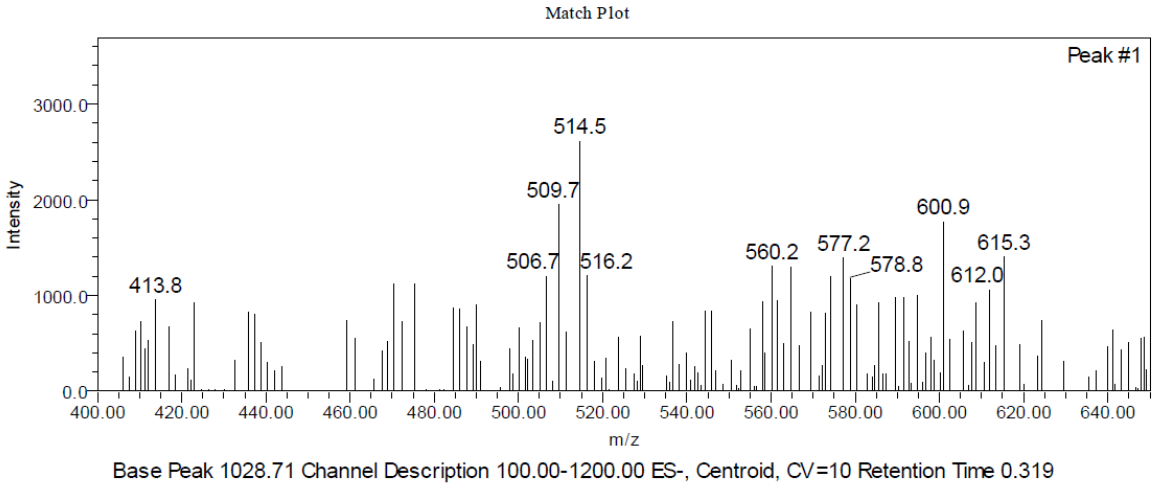


FIGURE 3.26: Mass spectra of 5p

3.2.4.17 1-(4-nitrobenzoyl)-3-(4-(2-oxo-2-(piperazin-1-yl)ethyl)phenylsulfonyl)guanidine(5q)

Reaction between 1-(4-Nitrobenzoyl) -3-(phenylsulfonyl) guanidine (0.1 mole) and 2-Chloro-1-(piperazin-1-yl)ethanone (0.1 mole) were performed in the presence of $FeCl_3$ and nitrobenzene. Yield 45%, m.p.: 90-92 °C. R_f value: 0.7 (mobile phase: ethyl acetate: hexane: 0.7:0.3)

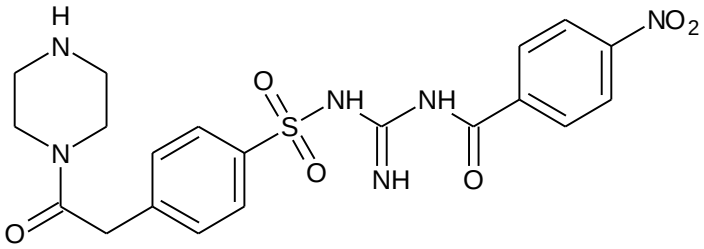


Table 3.19: Spectral data of 5q

Code	IR(KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5q	3453(N-H) *str*, 1640 (C=O) *str*, 1045 (S=O), 1378 (N-O) *str*, 1527 (N-O) *str*	475.2 [M]	8.022-8.053(d,4H,ArH), 7.35(d,4H,ArH),1.99(s,2H,NH), 1.11-1.22(t,4H,Piperazine), 1.31-1.77(t,4H,Piperazine).

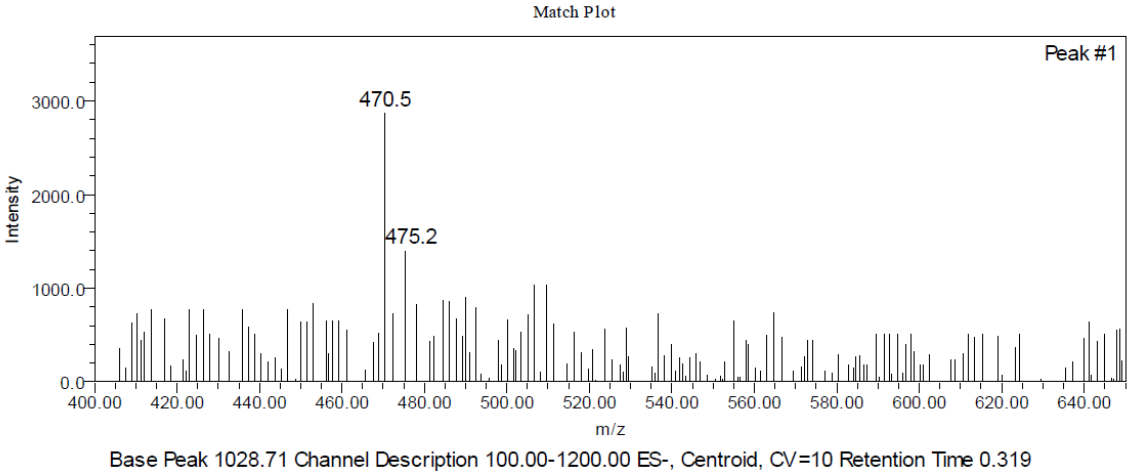


FIGURE 3.27: Mass spectra of 5q

General procedure for Synthesis of 1-(4-Substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea/gaunidine (5r-5w)

They were prepared by following the literature method. [48]

Friedel–Crafts alkylation of 1-(4-substitutedbenzoyl)-3-(phenylsulfonyl)urea/gaunidine (0.1 mmol) and 2-chloro-1-(pyrrolidin-1-yl)ethanone (1 mmol) was done for 7 hrs in the presence of $FeCl_3$ and Nitrobenzene as solvent. A reaction mixture was cooled and washes with ice cold water. Solid product was Recrystalized with rectified spirit.

3.2.4.18 1-(4-Methoxybenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1 yl)ethyl)phenylsulfonyl)urea(5r)

Yield: 65%, m.p. 150-152 °C; R_f = 0.75 (ethyl acetate: hexane 2:8 v/v)

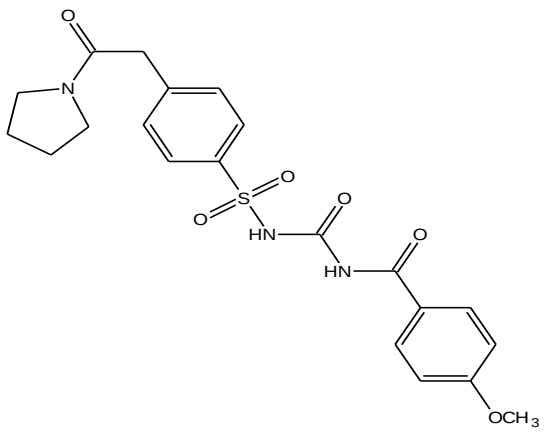


Table 3.20: Spectral data of 5r

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5r	3408(N-H) *str* 2850 (C-H) 1695 (C=O) 1259 (S=O) *str*	445 [M+1]	3.76(s,3H,OCH_3),7.87(d,4H,ArH),7.21(d,4H,ArH),10.18(s,1H,NH),2.34(t,4H,Pyrrolidine), 3.39(t,4H, Pyrrolidine)

3.2.4.19 1-(4-Fluorobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5s)

Yield: 75%; gray crystalline powder; m.p.=110ºC; R_f = 0.75 (ethyl acetate: hexane 2:8 v/v)

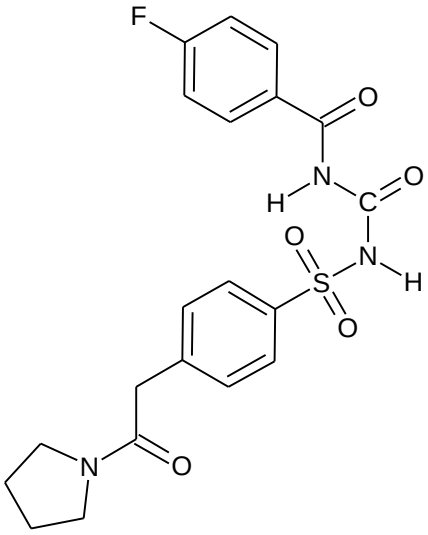


TABLE 3.21: Spectral data of 5s

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5s	3263 (N-H) 2973 (C-H) 1672 (C=O) *str* 1378 (S=O) 1150 (C-F)	435 [M+1]	7.87 (d, 4H, ArH), 7.25 (d, 4H, ArH), 10.03 (s, 1H, NH),2.14(t,4H,Pyrrolidine), 3.48(t,4H, Pyrrolidine)

3.2.4.20 1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5t)

Yield: 75%; yellow crystalline powder; m.p.=112-114 ºC; R_f = 0.60 (ethyl acetate: hexane 2:8 v/v

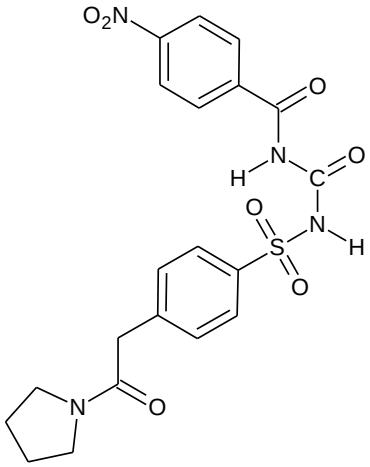


TABLE 3.22: Spectral data of 5t

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5t	3449 (N-H) *str* 2989 (C-H) 1680 (C=O) *str* 1187 (S=O) 1549 (N-O) 1318 (N-O) *str*	460 [M]	7.67 (d, 4H, ArH), 7.20 (d, 4H, ArH), 10.23 (s, 1H, NH),2.17(t,4H,Pyrrolidine), 3.28(t,4H, Pyrrolidine)

3.2.4.21 1-Benzoyl-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea(5u)

Yield: 65%; m.p.: 116-118°C. R_f = 0.60 (ethyl acetate: hexane 3:7 v/v)

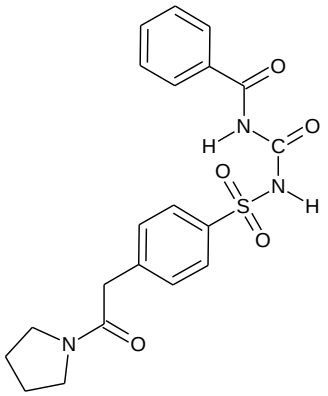


TABLE 3.23: Spectral data of 5u

Code	IR(KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5u	3274 (N-H) *str* 2993 (C-H) 1724 (C=O) *str* 1102(S=O) *str*	415.4 [M]	7.25 (d, 4H, ArH),7.49(s,1H,ArH),7.89 (d, 4H, ArH),2.17(t,4H,Pyrrolidine), 3.28(t,4H, Pyrrolidine)

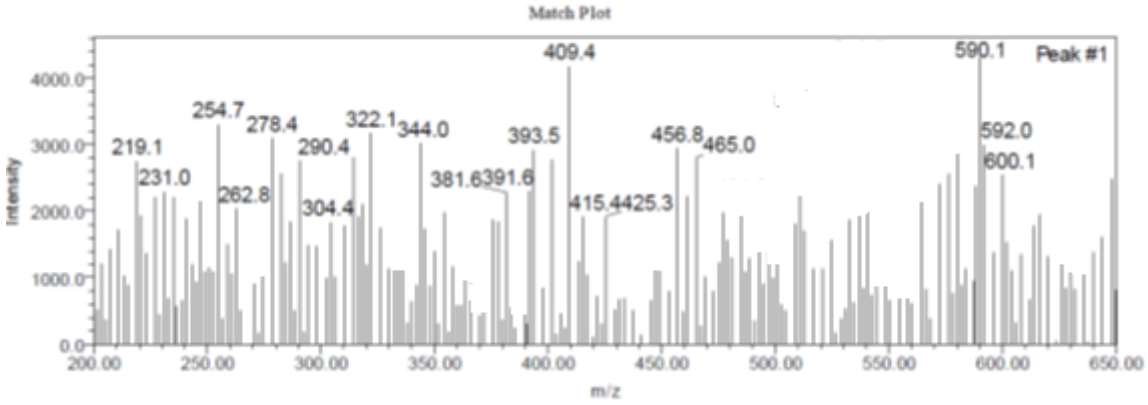


FIGURE 3.28: Mass spectra of 5u

3.2.4.22 1-(4-Chlorobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea (5v)

Yield: 70%; m.p.=122-126 °C. R_f value: 0.5 (ethyl acetate: hexane: 0.7:0.3)

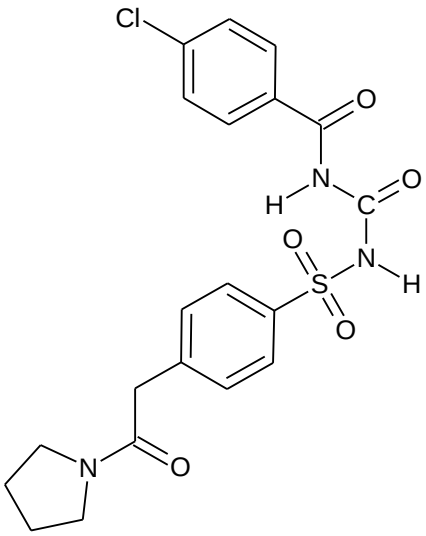


TABLE 3.24: Spectral data of 5v

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5v	3363 (N-H) *str* 2975(C-H) 1682(C=O) *str* 1378(S=O) *str* 850 (C-Cl)	452.9 [M+2]	7.87 (d, 4H, ArH), 7.25 (d, 4H, ArH), 10.03 (s, 1H, NH),1.39(t,4H,Pyrrolidine), 3.33(t,4H, Pyrrolidine)

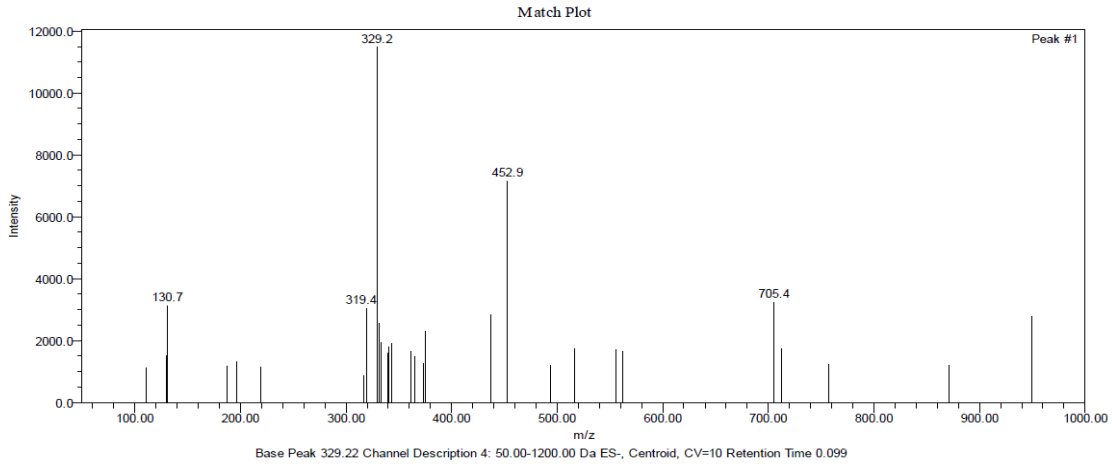


FIGURE 3.29: Mass spectra of 5v

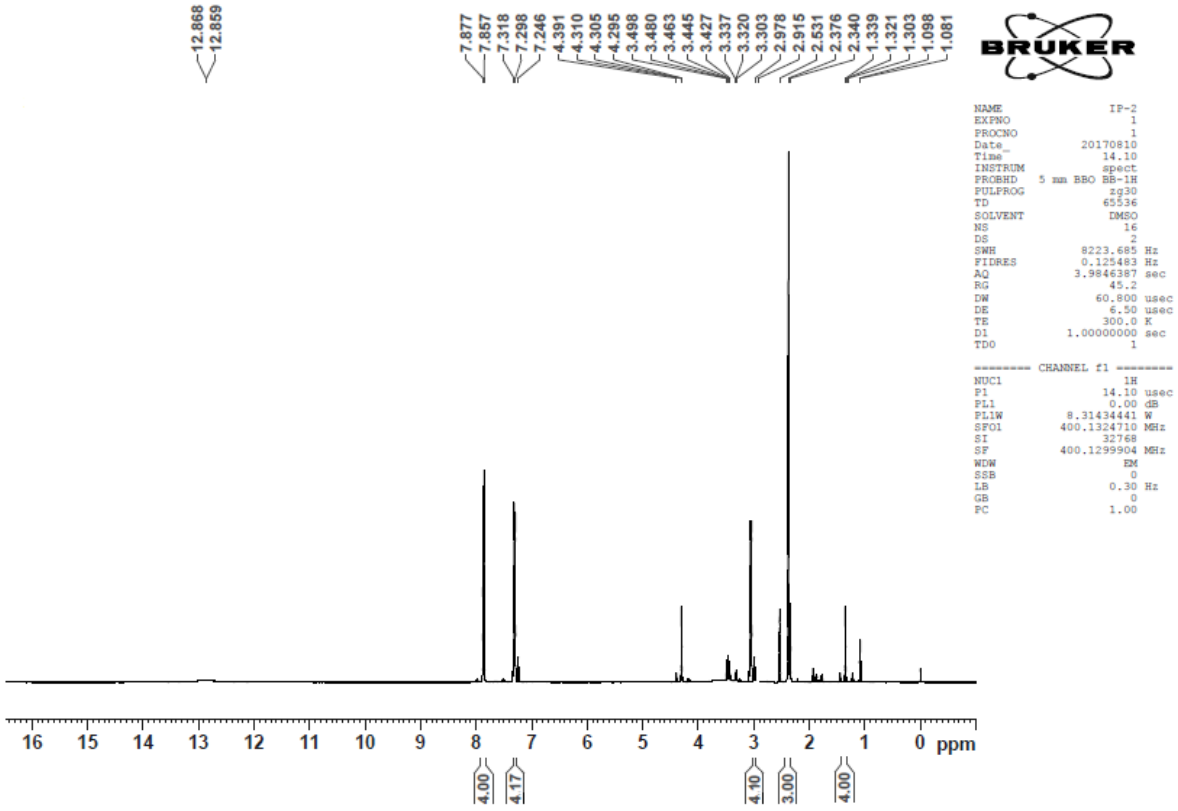


FIGURE 3.30: ^{1}H NMR of 5v

3.2.4.23 1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)guanidine(5w)

Yield: 70%; yellow crystalline powder; m.p.=106-108ºC; R_f = 0.60 (ethyl acetate: hexane 2:8 v/v

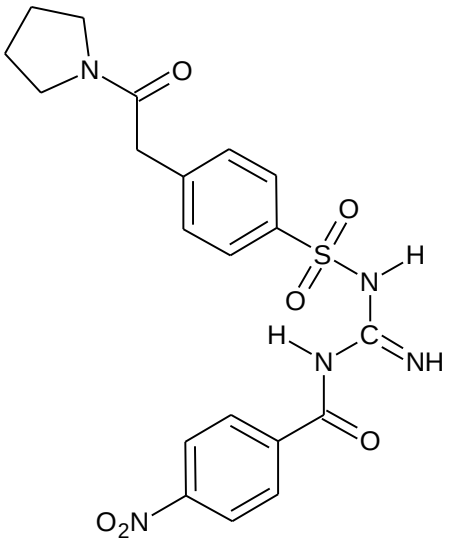


TABLE 3.25: Spectral data of 5w

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5w	3449 (N-H) *str* 2989 (C-H) 1680 (C=O) *str* 1187 (S=O) 1575,1336 (N-O) *str*,	459.3 [M]	7.67 (d, 4H, ArH), 7.20 (d, 4H, ArH), 10.23 (s, 1H, NH),2.17(t,4H,Pyrrolidine), 3.28(t,4H, Pyrrolidine),

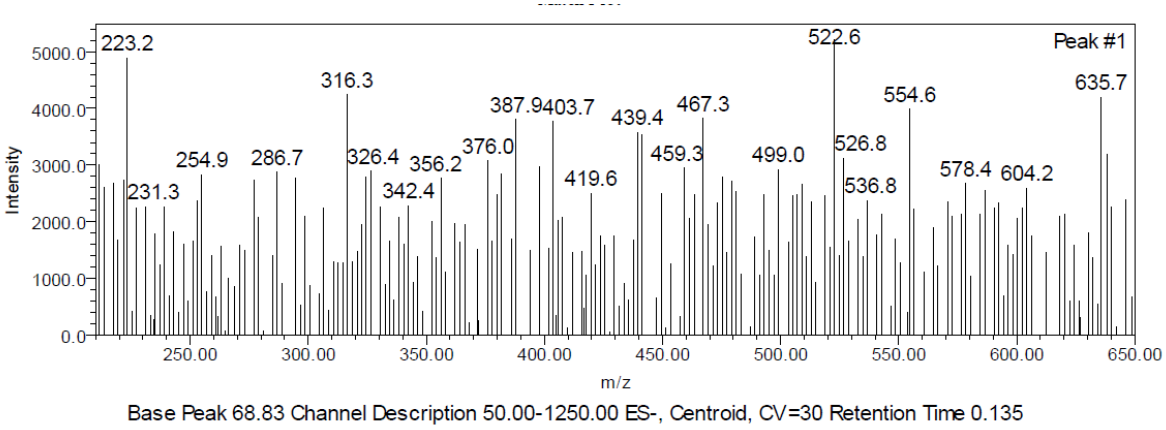


FIGURE 3.31: Mass analysis of 5w

General procedure for Synthesis of 1-(4-substitutedbenzoyl)-3-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)urea (5x-5J) were prepared by following the literature method.[48]

3.2.4.24 1-(4-(2-(4-Methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)gaunidine (5x)

Yield: 60%; m.p.= 90-94 °C. R_f value: 0.7 (mobile phase: ethyl acetate: hexane: 0.3:0.7)

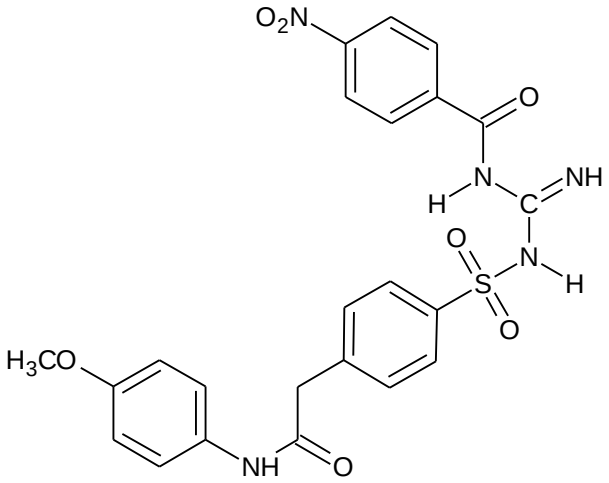


TABLE 3.26: Spectral data of 5x

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5x	3449 (N-H) *str* 2989 (C-H) 1684 (C=O) *str* 1187 (S=O) 1554,1320(N-O)	510.8 [M]	7.69 -7.21(d,4H,ArH),8.18-8.206(d,4H,ArH), 10.24(s,1H,NH),3.76(s,3H,OCH_3),3.062(s,2H,CH_2)

3.2.4.25 1-Benzoyl-3-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)phenylsulfonyl)urea (5y)

Yield: 60%; m.p.= 88-92 °C. R_f value: 0.6 (mobile phase: ethyl acetate: hexane: 0.3:0.7)

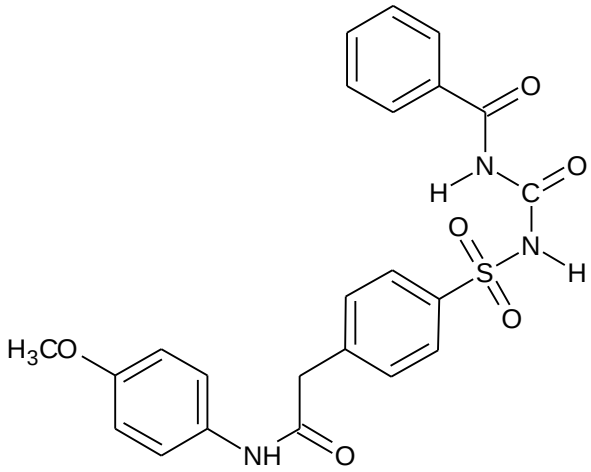


TABLE 3.27: Spectral data of 5y

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5y	3449 (N-H) *str*, 2989 (C-H) 1688 (C=O) *str* 1187 (S=O)	468.7 [M]	7.69-7.21(d,4H,ArH),8.18-8.206(d,4H,ArH), 10.24(s,1H,NH),3.76(s,3H,OCH_3),3.062(s,2H,CH_2)

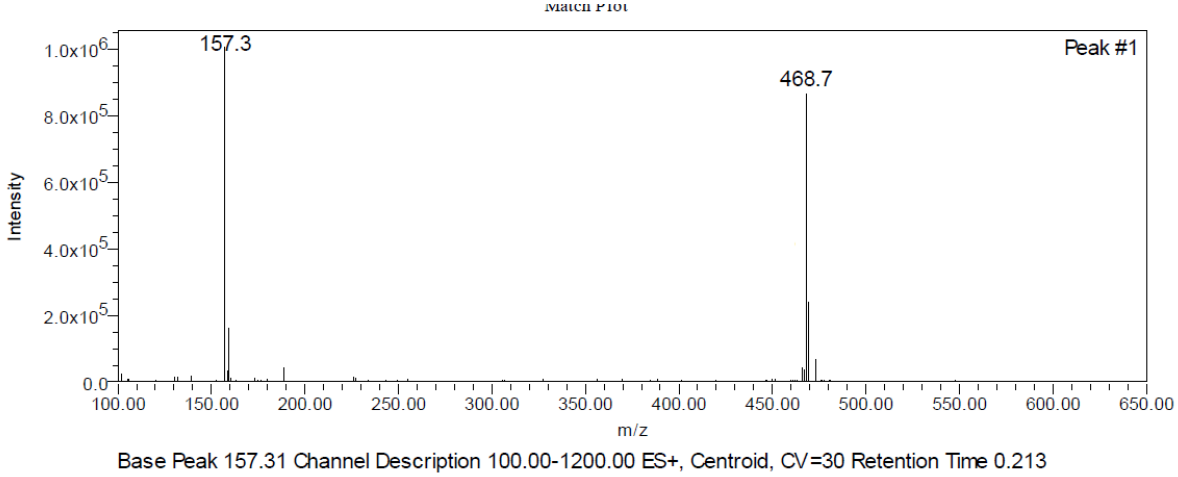


FIGURE 3.32: Mass analysis of 5y

3.2.4.26 1-(4-((4-Methoxyphenyl)carbamoyl)phenylsulfonyl)-3-(4-fluorobenzoyl)urea (5z) Yield: 55%; m.p.= 84-86 °C. R_f value: 0.55 (mobile phase: ethyl acetate: hexane: 0.3:0.7

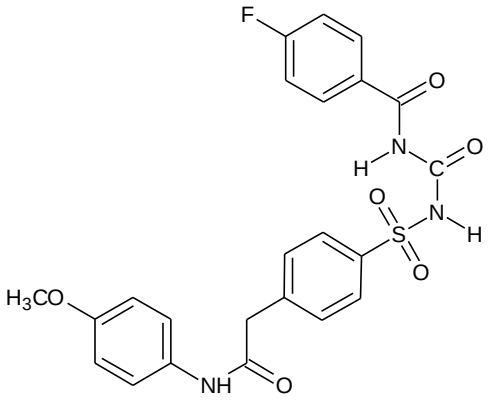


TABLE 3.28: Spectral data of 5z

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5z	3439 (N-H) *str* 2989(C-H) 1670(C=O) *str* 1187(S=O)	485 [M]	7.69-7.21(d,4H,ArH),8.18-8.206(d,4H,ArH), 10.24(s,1H,NH),3.76(s,3H,OCH_3),3.062(s,2H,CH_2)

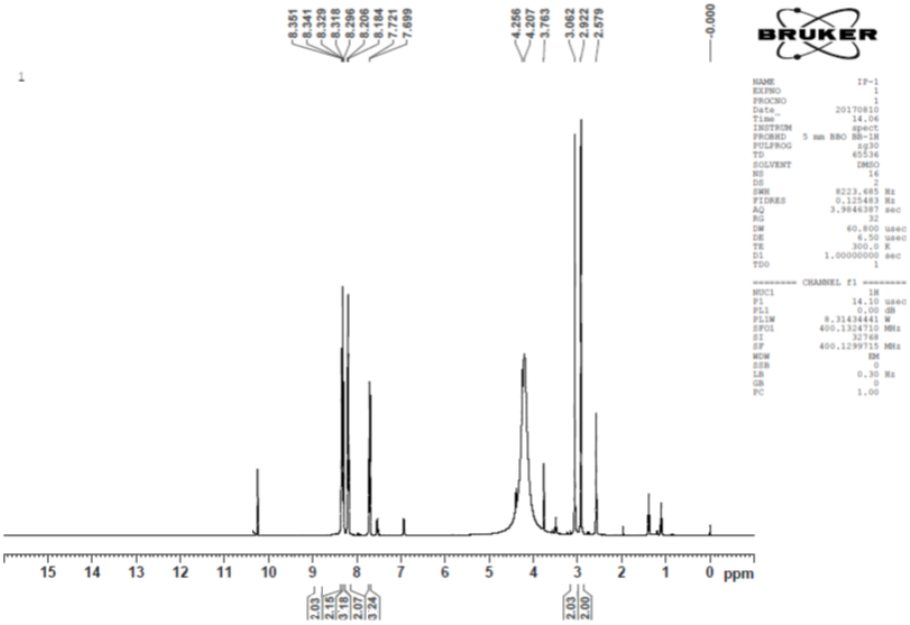


FIGURE 3.33: ^{1}H NMR of 5z

6.3.27 1-(4-Chlorobenzoyl)-3-(4-(2-(4-methoxyphenylamino) -2-oxoethyl)phenylsulfonyl)urea (5aa)

Yield: 42%; m.p.=84-86 °C. R_f value: 0.55 (mobile phase: ethyl acetate: hexane: 0.3:0.7)

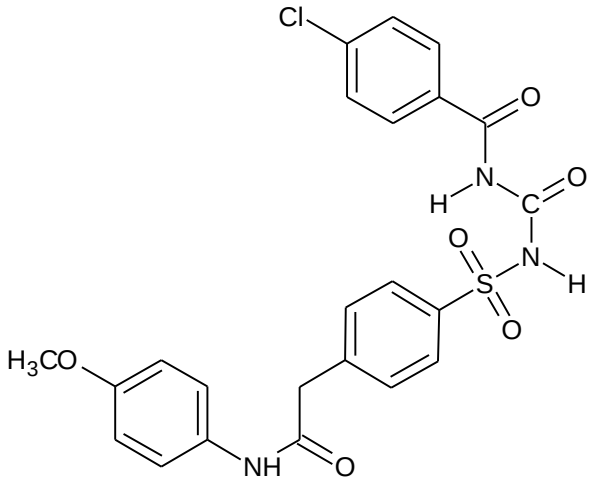


TABLE 3.29: Spectral data of 5aa

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5aa	3352 (N-H) *str* 2949 (C-H) 1690 (C=O) *str* 850 (C-Cl) 1167 (S=O) *str*	503 [M+1]	7.69-7.21(d,4H,ArH),8.18-8.206(d,4H,ArH), 10.24(s,1H,NH),3.76(s,3H,OCH_3),3.062(s,2H,CH_2)

3.2.4.28 1-benzoyl-3-(4-(2-oxo-2-(phenylamino)ethyl)phenylsulfonyl)urea (5ab)

Yield: 64%; m.p.=90-94 °C. R_f value: 0.55 (mobile phase: ethyl acetate: hexane: 0.3:0.7)

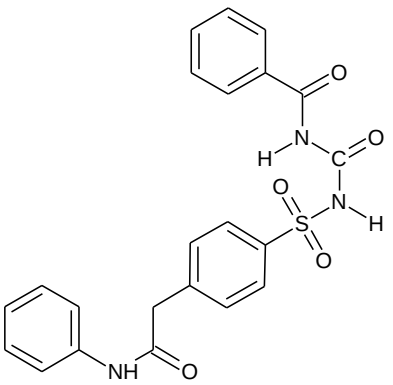


TABLE 3.30: Spectral data of 5ab

Code	IR (KBr): ν (cm^{-1})	MASS (m/z)	^{1}H NMR (400 MHz)
5ab	3352(N-H) *str*, 2949(C-H), 1690(C=O) *str*, 850(C-Cl), 1167(S=O) *str*	439.1 [M-1]	7.69-7.21(d,4H,ArH),8.18-8.206(d,4H,ArH), 10.24(s,1H,NH),3.76(s,3H,OCH_3),3.062(s,2H,CH_2)

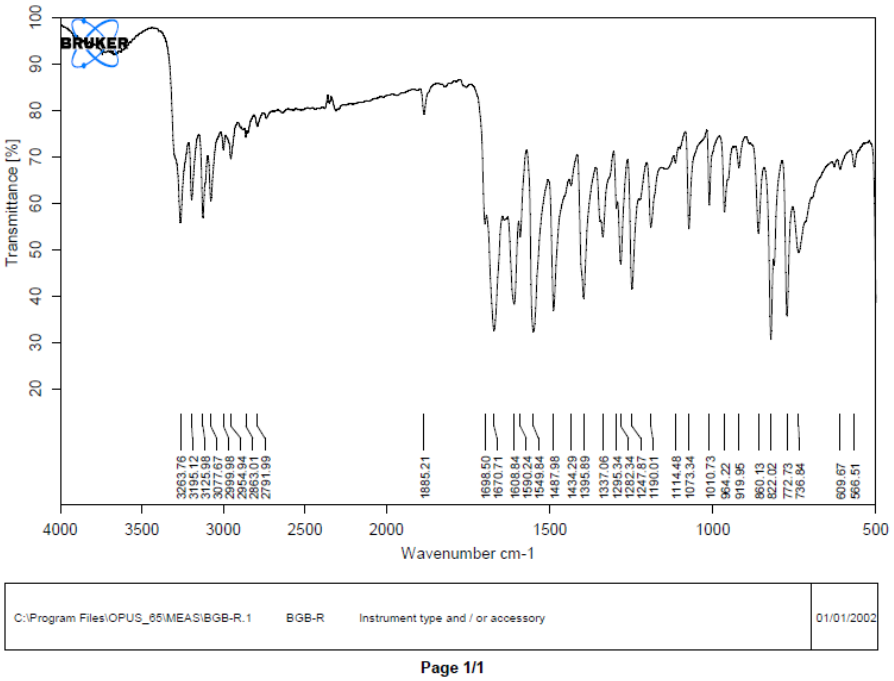


FIGURE 3.34: IR spectra of 5ab

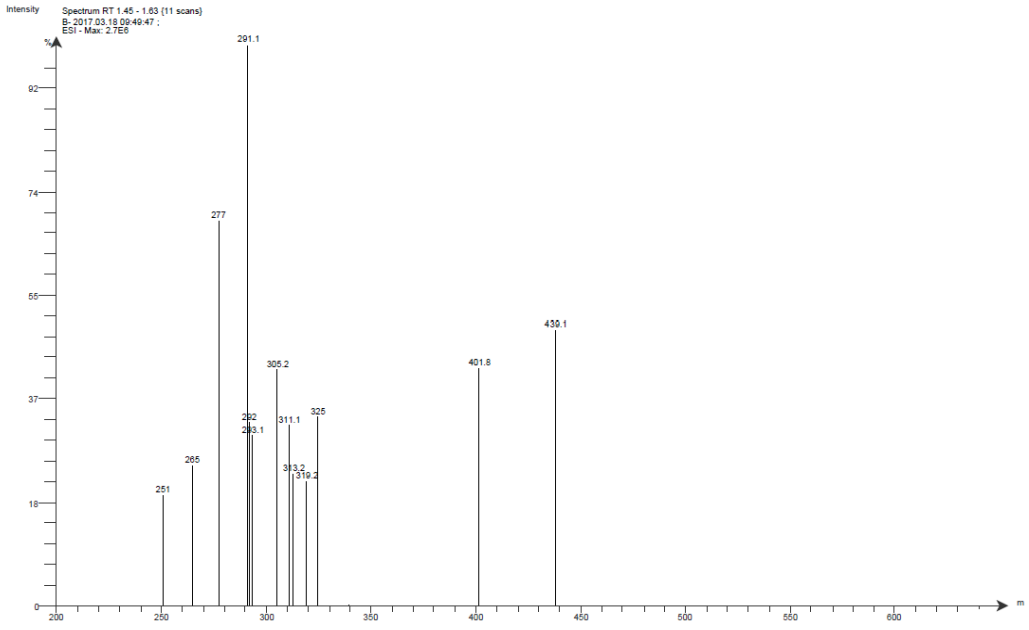


FIGURE 3.35: Mass spectra of 5ab

3.5 *In-vivo* Biological Evaluation

3.5.1 Preparation of diabetic animals

Rats of wistar strain were procured from the animal house, department of pharmacology, Parul institute of pharmacy, Parul University, Vadodara. They were used in this study. Experiments were carried out in male rats weighing between 150 g and 200 g. They were housed (six per cage) in plastic cages (47cm×34cm×18cm) lined with husk renewed every 24 hrs. The rats were fed on a pellet diet (Hindustan Lever, India) and drinking water.

Diabetes was induced in the rats by a single intraperitonial injection of alloxan (150 mg/kg body weight). Since alloxan is capable of producing fatal hypoglycemia as a result of the massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitonial after 6 h. The rats were then kept for the next 24 h on 5% glucose solution to prevent hypoglycemia. After 1 week, rats with moderate hyperglycaemia with blood glucose range of 200–400 $mgdl^{-1}$ were used for the study. Blood was collected from the tail vein. All of the target molecules were given to the diabetic rats orally in the form of suspension in carboxy methyl cellulose.

The animals were housed under standard laboratory conditions maintained at $25\pm10^{o}C$ and under 12/12 hour light/dark cycle. The experimental protocol was approved by the institutional animal ethics committee (Protocol No: PIPH 03/16) and by the animal regulatory body of the Indian Government (Registration No: 921/PO/EReBi/S/05/CPCSEA/PIPH03).

3.5.2 Experimental design

Diabetes was induced in rats, 1 week before starting the treatment. The rats were divided into seventeen groups as follows, after the induction of alloxan (150 mg/kg, intraperitonial) diabetes and each containing six rats, animals with blood glucose levels between 200-400 mg/dl were divided into the following groups.

Group 1: Normal -Normal controlled rats fed with 0.5 ml of normal saline.

Group 2: Diabetic control (DC) rats; fed with 0.5ml of normal saline.

Group 3: Diabetic rats treated with standard drug Glibenclamide 5 mg/kg body weight

Group 4: Diabetic rats; treated with synthesized drug 5a in 1% CMC 50 mg/kg of body weight.

Group 5: Diabetic rats; treated with synthesized drug 5c in 1% CMC 50 mg/kg of body weight.

Group 6: Diabetic rats; treated with synthesized drug 5d in 1% CMC 50 mg/kg of body weight.

Group 7: Diabetic rats; treated with synthesized drug 5f in 1% CMC 50 mg/kg of body weight.

Group 8: Diabetic rats; treated with synthesized drug 5h in 1% CMC 50 mg/kg of body weight.

Group 9: Diabetic rats; treated with synthesized drug 5i in 1% CMC 50 mg/kg of body weight.

Group 10: Diabetic rats; treated with synthesized drug 5m in 1% CMC 50 mg/kg of body weight.

Group 11: Diabetic rats; treated with synthesized drug 5n in 1% CMC 50 mg/kg of body weight.

Group 12: Diabetic rats; treated with synthesized drug 5o in 1% CMC 50 mg/kg of body weight.

Group 13: Diabetic rats; treated with synthesized drug 5p in 1% CMC 50 mg/kg of body weight.

Group 14: Diabetic rats; treated with synthesized drug 5q in 1% CMC 50mg/kg of body weight.

Group 15: Diabetic rats; treated with synthesized drug 5s in 1% CMC 50 mg/kg of body weight.

Group 16: Diabetic rats; treated with synthesized drug 5x in 1% CMC 50 mg/kg of body weight.

Group 17: Diabetic rats; treated with synthesized drug 5z in 1% CMC 50 mg/kg of body weight.

The dose for the newly synthesized compounds was decided on the basis of literature survey. [49] Glibenclamide was taken as the standard. The blood glucose level was determined at 0 and 3 hours after administration of test compounds using glucometer (Johnson & Johnson Pvt. Ltd.) Percentage reduction in plasma glucose level was calculated for each animal.

3.5.3 Statistical Analysis

Measurement data were tabulated as means ± S. E. M. Comparision were carried out using one way analysis of variances (ANOVA) followed by post-hoc Tukey test and *p-value<0.01 as the level of significance. Data was analyzed using the Graph Pad Prism 5.3, San Diego, CA.

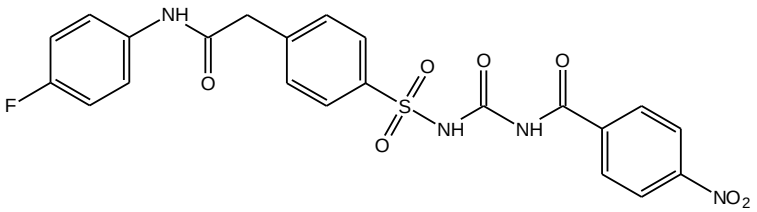
CHAPTER 4

Results and Discussions

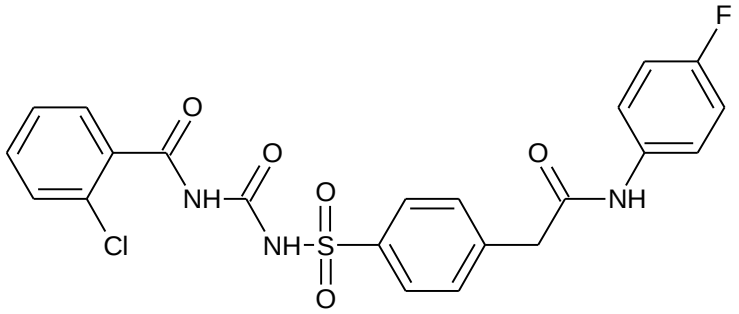
4.1 Molecular docking studies

The results were obtained as docking score, i.e. binding energy which is mentioned in **Table 4.1**

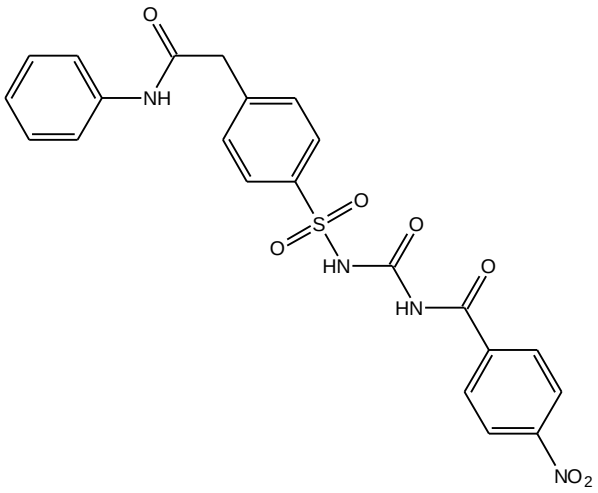
The entire designed molecules have shown a good binding affinity in comparison with standard glibenclamide. Among 28 various substituted sulphonylureas and guanidine derivatives, compound 5c, 5n, 5f, 5i, 5p and 5z showed a better binding affinity in comparison with glibenclamide. The 1-(4-(2-(4-Fluorophenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)urea (5c) contain NO_2 and F group at para position of benzene ring.



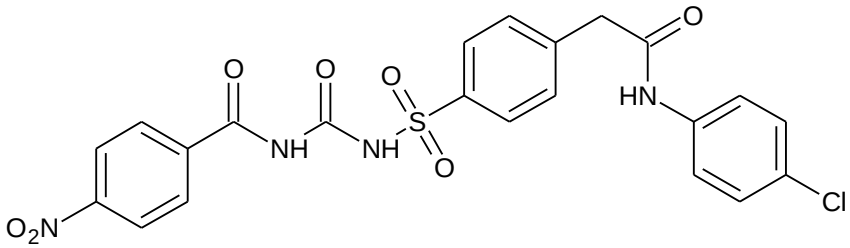
The 1-(2-Chlorobenzoyl)-3-(4-(2-(4-fluorophenylamino)-2-oxoethyl)phenylsulfonyl)urea (5f) also have electronegative groups (Cl, F) at the para position.



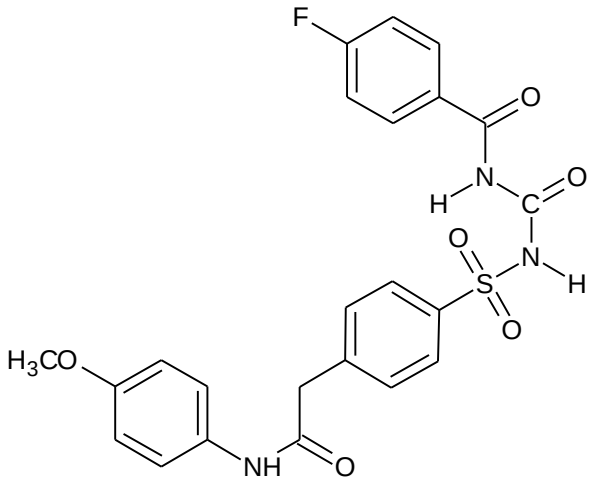
The 1-(4-Nitrobenzoyl)-3-(4-(2-oxo-2-(phenylamino) ethyl) phenylsulfonyl)urea (5i) have NO_2 group in the para position of the phenyl ring.



The 1-(4-(2-(4-chlorophenylamino)-2-oxoethyl)phenylsulfonyl)-3-(4-nitrobenzoyl)urea (5p) has Nitro and Chloro like electron withdrawing groups on the phenyl ring.



The 1-(4-((4-Methoxyphenyl) carbamoyl) phenylsulfonyl)-3-(4-fluorobenzoyl)urea (5z) has methoxy and F at the para position of the phenyl ring.



So, with the help of docking studies, we can say the electron withdrawing groups has significants effects on binding with receptor. As well as pyrrolidine ring does not have any notable affinity with binding pokect. **(5r-5w)**

Binding cavity and interaction with the various amino acid residues with the compound 5s,5d,5c,5ab and 5e were shown in Figure 4.1 to 4.5

TABLE 4.1: Docking results of the designed compounds

Code	Docking score	H- bond energy	Vander Waal energy
5a	-112.311	- 16.3875	-95.9239
5b	-105.994	-13.5407	-92.4534
5c	**-120.836**	**-23.6739**	**-97.1619**
5d	-117.585	-17.7774	-99.8077
5e	-114.053	-15.1444	-98.9087
5f	**-129.498**	**-17.2181**	**-112.28**
5g	-109.071	-9.57071	-99.5003
5h	-119.895	-10.5	-109.395
5i	**-120.41**	**-12.5227**	**-107.887**
5j	-108.041	-35.4247	-73.5922
5k	-105.871	-8.52901	-97.3424
5l	-106.453	-21.6474	-84.8055
5m	-114.529	-20.3845	-94.145
5n	**-124.972**	**-13.2616**	**-111.71**
5o	-112.428	-10.2911	-102.137
5p	**-123.115**	**-16.6997**	**-105.665**
5q	-116.077	-25.9192	-90.1581
5r	-104.667	-95.0936	-19.5733
5s	-108.3982	-86.3291	-13.0691
5t	-104.018	-71.8192	-33.386
5u	-99.6336	-90.4049	-9.22871
5v	-102.399	-91.801	-10.5978
5w	-107.77	-89.3005	-19.6134
5x	-114.205	-82.6018	-23.6027
5y	-112.783	-94.8273	-17.9556
5z	**-118.063**	**-105.333**	**-12.7301**
5aa	-104.372	-98.9745	-5.39719
Glibenclamide	**-108.996**	**-91.48**	**-17.5158**

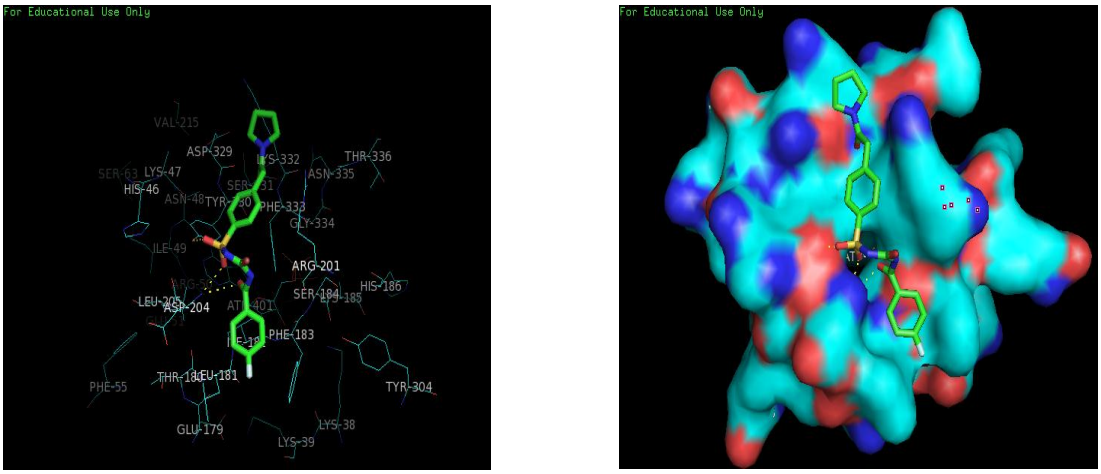


FIGURE 4.1 Binding pocket and interaction with various residues of compound 5s

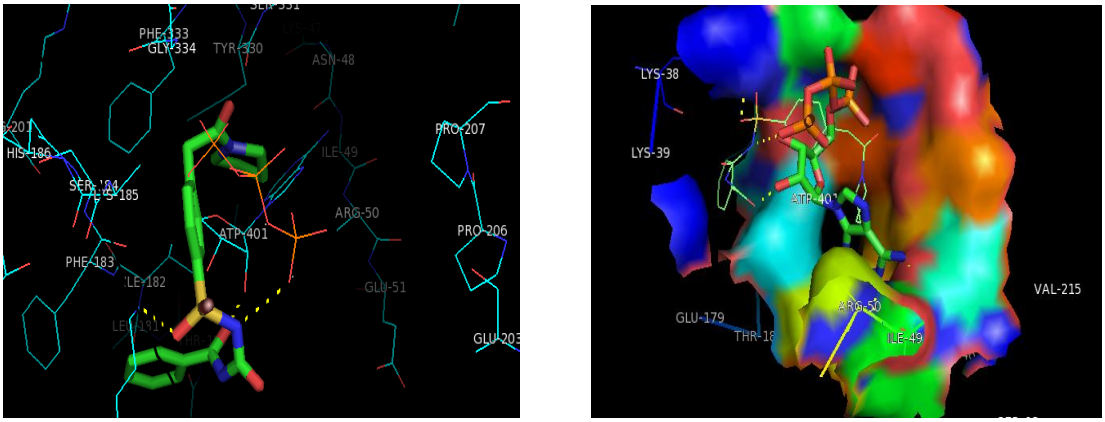


FIGURE 4.2 Binding pocket and interaction with various residues of 5d

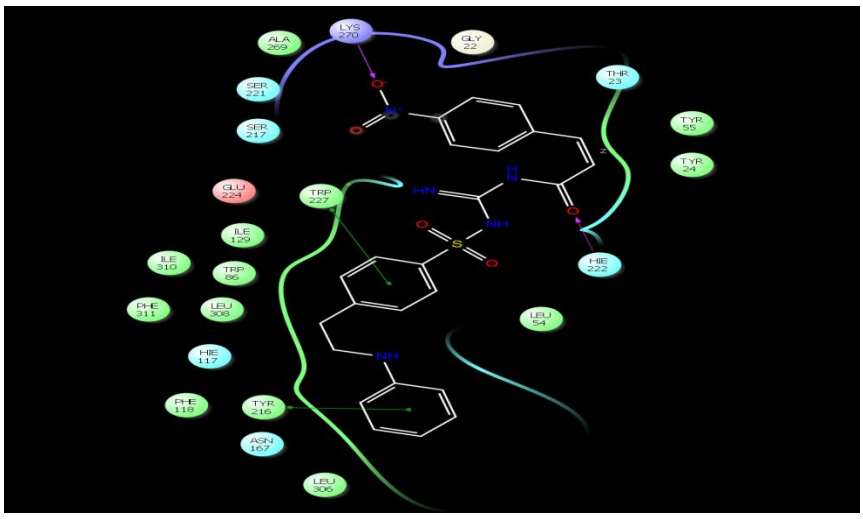


FIGURE 4.3 Binding pocket and interaction with various residues of 5e

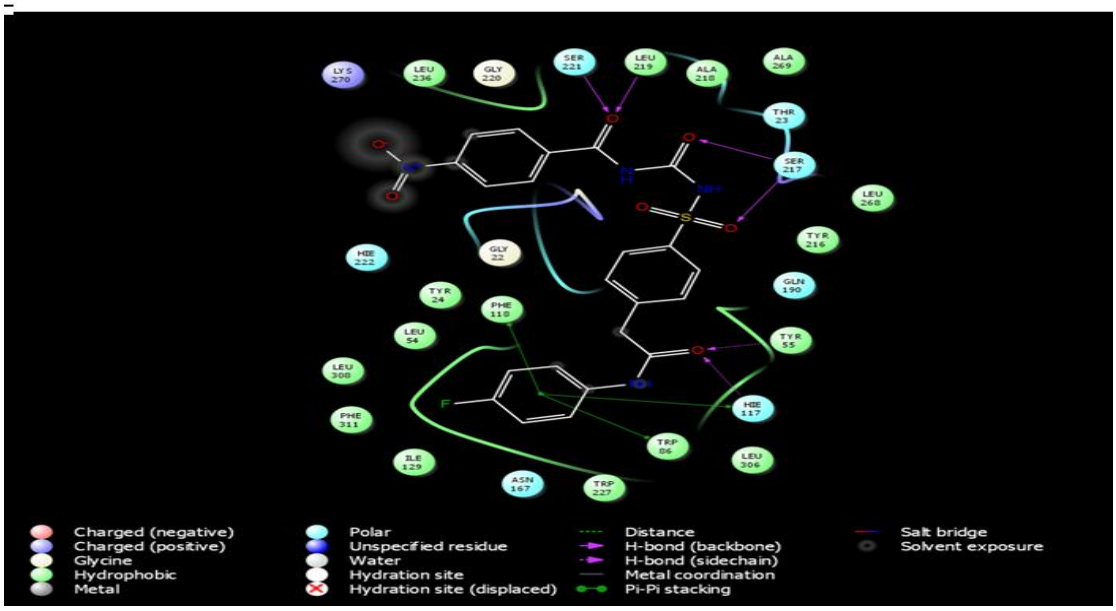


FIGURE 4.4 Binding pocket and interaction with various residues of 5c

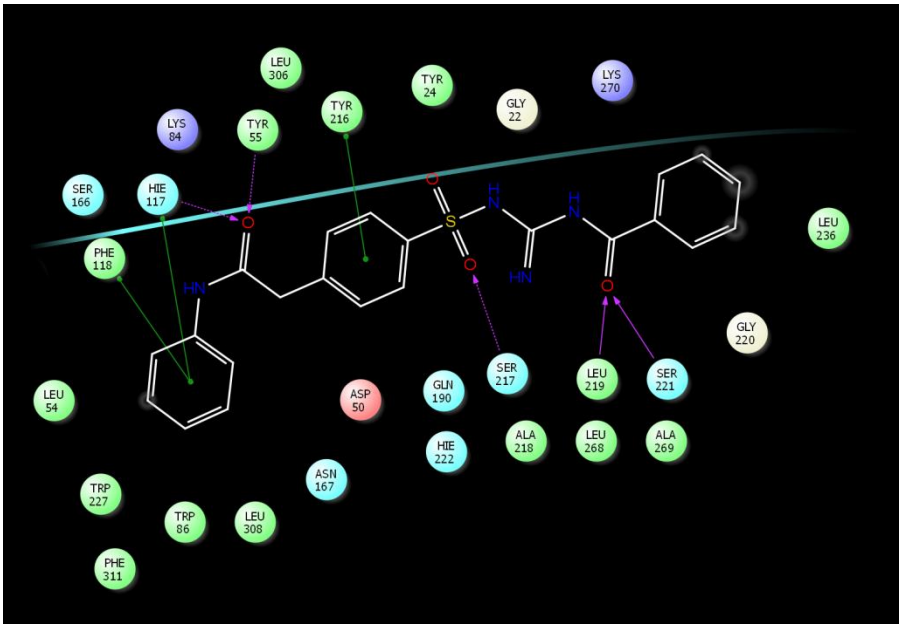


FIGURE 4.5 Binding pocket and interaction with various residues of 5ab

4.2 In-*silico* toxicity studies

In-silico toxicity profile of designed molecules was done using the SWISS ADME programme. The Lipnski rule of five was applied. The acceptability of analogues based on Lipinski's rule of five which was essential to ensure drug like properties. All the design and synthesized derivatives follow the Lipinski rule of five. Most of the compounds have drug like property with good GI absorption. The results of *in-silico* toxicity studies mentioned in **Table4.2.**

TABLE 4.2: *In-silico* toxicity studies

Code	Mol. wt (g/mol)	HBd	HBa	C Log P	Drug likeness
5a	459	**4**	**7**	**2.15**	Yes, 0 violations
5b	457	3	5	2.91	Yes, 0 violations
5c	500	4	5	2.20	Yes, 0 violations
5d	471	3	5	2.88	Yes, 0 violations
5e	476	4	6	2.88	Yes, 0 violations
5f	489	3	6	3.17	Yes, 0 violations
5g	488	4	6	3.60	Yes, 0 violations
5h	454	4	6	2.88	Yes, 0 violations
5i	484	4	7	1.803	Yes, 0 violations
5j	502	5	7	3.591	Yes, 1 violation
5k	430	3	6	1.621	Yes, 0 violations
5l	448	3	7	1.7	Yes, 0 violations
5m	488	3	7	3.60	Yes, 0 violations
5n	489	3	6	3.17	Yes, 0 violations
5o	489	4	6	3.60	Yes, 0 violations
5p	517	4	7	2.77	Yes; 1 violation
5q	475	5	8	1.793	Yes, 0 violations
5r	445	2	6	2.44	Yes, 0 violations
5s	433	2	6	2.66	Yes, 0 violations
5t	461	3	7	2.26	Yes, 0 violations
5u	415	2	5	2.50	Yes, 0 violations
5v	449	2	5	2.11	Yes, 0 violations
5w	460	7	10	2.69	Yes, 0 violations
5x	513	4	8	2.54	Yes, 1 violation
5y	467	3	6	2.32	Yes, 0 violations
5z	485	3	7	2.65	Yes, 0 violations
5aa	501	3	6	2.85	Yes, 0 violations
5ab	437.	2	6	2.76	Yes, 0 violations

4.3 Sci finder report

Here Sci finder report of some synthesized derivatives is given. In report it is mentioned that resulting 0 candidates it indicate our compounds are novel.

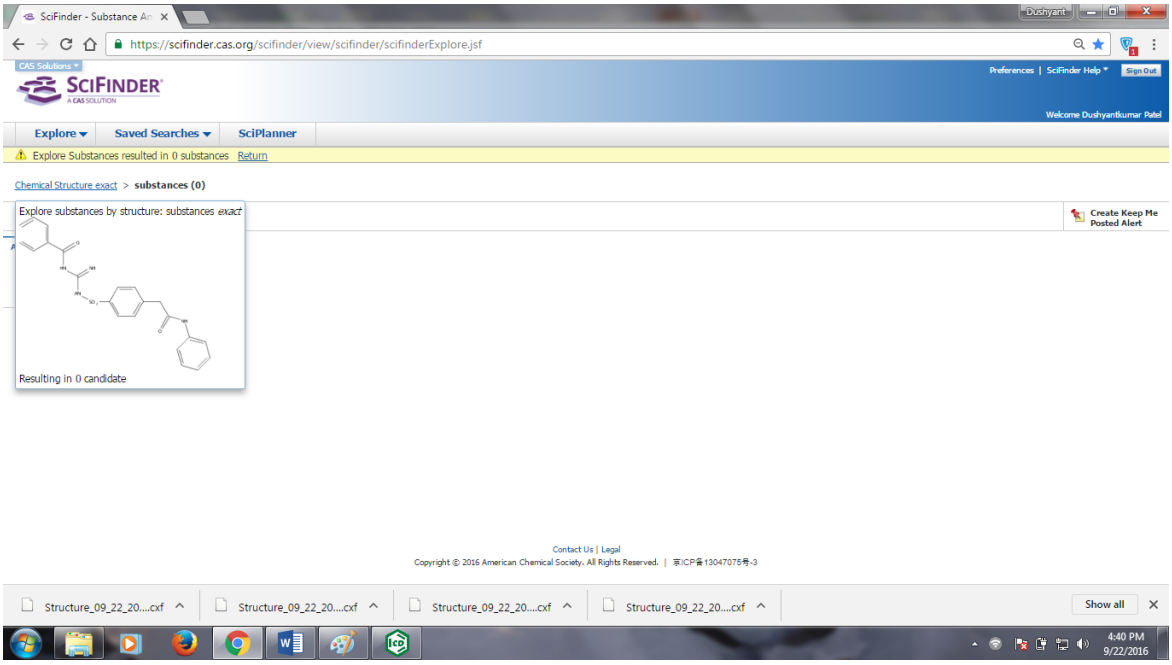


FIGURE 4.6 Sci finder report of 5ab

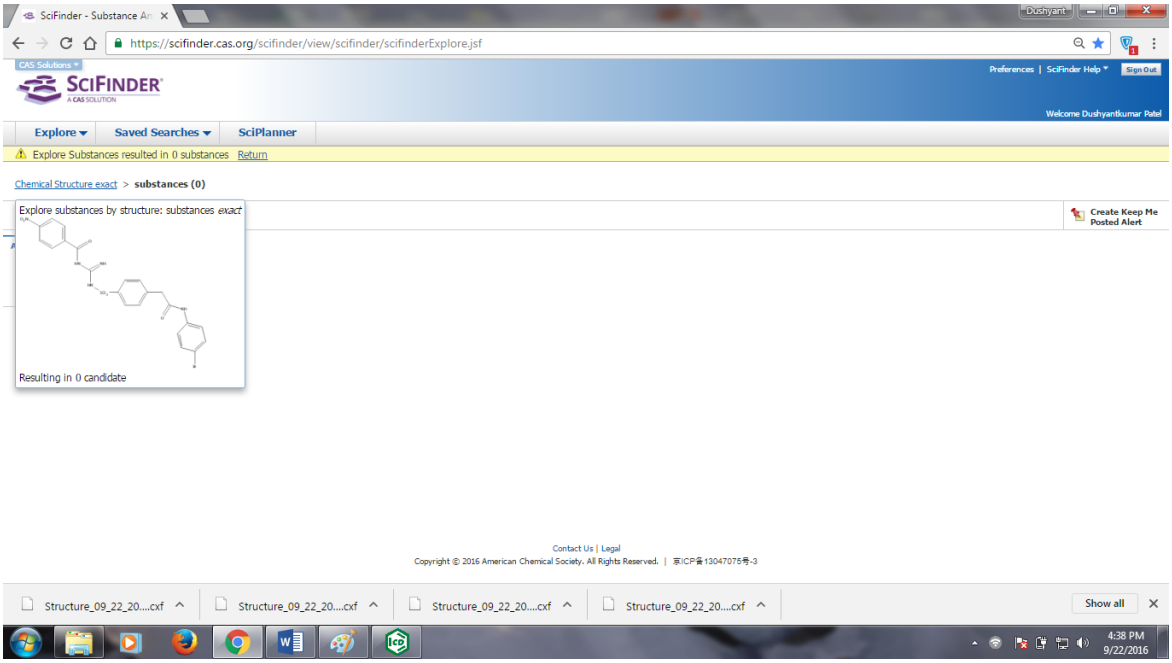


FIGURE 4.7 Sci finder report of 5c

4.4 Experimental section

4.4.1 Chemistry – Scheme 1, 2, 3

General procedure for Synthesis of 1-(4-((4-substitutedphenyl)carbamoyl) phenylsulfonyl)-3-(4-substitutedbenzoyl)urea/gaunidine were synthesize using literature method. [48] Friedel Craft alkylation of 1-(4-substitutedbenzoyl)-3-(phenylsulfonyl) urea (0.1 mmol) and N-(4-substitutedphenyl)-2-chloroacetamide (1 mmol) was done in 7 hrs in the presence of anhydrous $FeCl_3$. Nitrobenzene was taken as solvent. A reaction mixture was cooled and washed with ice cold water. Solid product was recrystallized by rectified spirit.

The synthesis compound **1** was done by reacting benzene sulphonyl chloride with excess amount of urea/guanidine under reflux condition for 5 hrs. Pyridine (0.2 ml) was taken as catalyst. Reaction mixture was monitored by thin layer chromatography. Different derivatives (NO_2, OCH_3, Cl and F) of benzene carboxylic acid were converted into benzene carbonyl chloride **2** with the help of $SOCl_2$ under reflux condition for 3 hrs. Compound **1** was dissolved in absolute alcohol and reacted with compound **2** under reflux condition for 2 hrs. Pyridine (0.2 ml) was taken was the catalyst. Reaction mixture was decanted into ice cold water, solid product was isolated and recrystallizes with rectified spirit. Different 2^{nd} and 4^{th} substituted (F, Cl, NO_2, Br) aniline derivatives, Pyrrolidine, piperazine derivatives was stirred with chloro acetyl chloride under cooling condition for 4 hrs in fuming hood. After addition of ice cold water in a reaction mixture solid crystals of N-(4-substitutedphenyl)-2-chloroacetamide **4** were isolated.

One step Friedel Crafts alkylation was performed to prepare1-(4-substitutedbenzoyl)-3-(4-(2-oxo-2-(pyrrolidin-1-yl)ethyl)phenylsulfonyl)urea/guanidine (5a-5ab) as illustrated in scheme (1), (2), and (3) an suitable 1-(4-substitutedbenzoyl)-3-(phenylsulfonyl)urea/guanidine and 2 N-(4-substitutedphenyl)-2-chloroacetamide. Nitrobenzene was used as solvent and anhydrous $FeCl_3$ act as catalyst. Reflux was done for 6 to 7 hrs and the mixture was poured in ice cold water to get final product **5**. The target compounds **5a-5ab** was purified by recrystallization. Reaction of all targeted molecules was monitored by thin layer chromatography. Ethyl acetate and hexane (3:7) was used as mobile phase. Some impurities were very close to most of our compounds, which leads to obtain a less percentage yield of pure target compounds.

4.4.2 Spectral characterization

Synthesized compounds were identified Infra-red spectroscopy, Mass spectroscopy, and ^{1}H and ^{13}C Nuclear Magnetic Resonance for all target compounds. In Mass spectroscopy, we got M (5c,5o,5p, 5q,5t, 5u, 5w, 5x,5y,5z), M+1 (5a, 5d, 5e, 5g, 5h, 5k, 5l,5r,5s), M-1 (5f,5j,5m, 5n,5ab), M-2 (5b,5i), and M+2 (5v,5aa) for targeted derivatives. M+ 2 peaks obtained because of Cl (5v and 5aa) atom.

In ^{1}H-NMR doublet was obtained for aromatic hydrogen between 7 to 8.5 delta values. In the same way singlet of NH of urea/guanidine derivatives is obtained between 7.98 to 10.41.

The absorption band at 1670 cm^{-1} to1724 cm^{-1} corresponds to C=O is stretching of carbonyl and amide for targeted compounds (5a-5ab). Also N-O stretching was observed between 1350 cm^{-1} and 1568 cm^{-1} (5c,5i,5j,5p,5q,5t,5w,5x). Halogen stretching of C-F was present between 1113 cm^{-1} and 1314 cm^{-1} (5f, 5g, 5h, 5l, 5s). Likewise stretching of C-Cl was observed between 650 cm^{-1} to 822 cm^{-1} (5d, 5p, 5v, 5aa).

4.5 Biological evaluation

Molecular targets of compounds:

The insulin is amide linkage hormone and my products are sulphonylureas so NHCONH linkage is present and peptide hormone insulin has NHCO so it can easily bind to insulin receptors. The sulfonylurea receptors (SUR) are membrane proteins which are the molecular targets of the sulfonylurea class of antidiabetic drugs whose mechanism of action is to promote insulin release from pancreatic beta cells. More specifically, SUR proteins are subunits of the inward-rectifier potassium ion channels Kir6.x (6.1 and 6.2). The association of four Kir6. x and four SUR subunits form an ion conducting channel commonly referred to as the KATP channel.

DOES selection of compounds is done by literature review for biological evaluation of hypoglycemic agents. S Prakash, D Maji, S Samanta and RK Sinha has reported Design, Synthesis and Antidiabetic, Cardiomyopathy Studies of Cinnamic Acid-Amino Acid Hybrid Analogs, *Med chem*, 4, 2, 1-6. With references of this article I have selected this does.

Plasma concentrations or bioavailability of the compounds were not measured. We performed primary screening of the synthesized compounds to identify most active derivatives. Further pharmacological evaluation was not performed as it was not relevant to the objective of the problem. However, fact that it produces the pharmacological action after oral administration it implies that it was bioavailable.

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia. The ability of target molecules to bind with sulphonylureas receptor was resolute by testing them at an albino wistar rate for measurement of reduction of blood sugar level. Blood Data analysis was done by graph pad prism one way ANOVA followed by turkey test. In our research, we have found that administration of compounds to diabetic rats reversed their blood glucose. The possible mechanism by which they brings about them hypoglycemic action may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form.

However, the Compound **5c (50.88±3.7), 5n (47.93±5.4), 5o (45.27±5.2), 5x (41.39±3.7), 5z (41.52±5.9)** derivatives of sulfonylureas showing better % reduction of blood glucose level (Table no 4.3, 4.4 and Figure-4.4) compare to other derivatives. **5c, 5x** contain electronegative atom (NO_2) in the 4^{th} position of benzene ring which has significant effects on blood sugar reduction. In another side **5z, 5n**, and **5o** contains Cl and F functional groups respectively in the 4^{th} position of sulfonylureas derivatives, which was showing better blood sugar reduction compare to other derivatives.

TABLE 4.3: One way Anova followed by turkey test

Bartlett's statistic (corrected)	33.26				
P value	0.0043				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	**SS**	**df**	**MS**		
Treatment (between columns)	15691	15	1046		
Residual (within columns)	24149	96	251.6		
Total	39840	111			
Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**Significant? P < 0.01?**	**Summary**	**99% CI of diff**
DC vs Gli	-57.26	9.552	Yes	***	-91.62 to -22.90
DC vs 5a	-35.28	5.885	Yes	**	-69.64 to -0.9166
DC vs 5c	-50.25	8.383	Yes	***	-84.61 to -15.89
DC vs 5d	-43.09	7.189	Yes	***	-77.46 to -8.733
DC vs 5f	-37.94	6.329	Yes	**	-72.30 to -3.577
DC vs 5h	-32.18	5.368	No	*	-66.54 to 2.182
DC vs 5i	-34.39	5.737	Yes	**	-68.75 to -0.02902
DC vs 5m	-37.34	6.229	Yes	**	-71.70 to -2.980
DC vs 5n	-47.30	7.890	Yes	***	-81.66 to -12.94
DC vs 5o	-44.65	7.448	Yes	***	-79.01 to -10.28
DC vs 5p	-38.50	6.423	Yes	**	-72.86 to -4.142
DC vs 5q	-37.41	6.241	Yes	**	-71.77 to -3.049
DC vs 5s	-31.04	5.177	No	*	-65.40 to 3.325
DC vs 5x	-40.76	6.800	Yes	***	-75.12 to -6.400
DC vs 5z	-40.89	6.821	Yes	***	-75.25 to -6.525

TABLE 4.4: In-vivo biological activity

Groups	Mean± S.E.M
DC	0.6287±0.9512
GLI	57.89±4.905
5a	35.91±2.9
5c	50.88±3.7
5d	43.72±10.6
5f	38.57±5.1
5h	32.81±5.3
5i	35.02±8.7
5m	37.97±8.02
5n	47.93±5.4
5o	45.27±5.2
5p	39.13±5.1
5q	38.04±6.024
5s	31.66±7.02
5x	41.39±3.7
5z	41.52±5.9

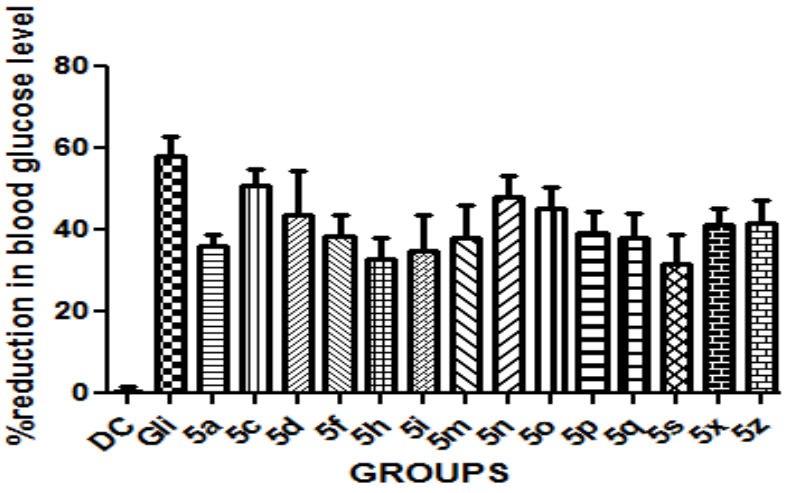


FIGURE 4.8 In *vivo* biological evaluations

CHAPTER 5

Summary and Conclusion

5.1 Summary of present work

5.1.1 Molecular docking studies

The results were obtained as docking score, i.e. binding energy which is mentioned in **Table 4.1**The entire designed compound has shown a good binding affinity in comparison with standard glibenclamide, out of this Compound 5c (-120.836), 5n (-124.972), 5f (-129.498), 5i (-120.41), 5p (-123.115) and 5z (-118.063) showed a better binding affinity in comparison with glibenclamide.

5.1.2 *In-silico* toxicity studies

In-silico toxicity profile of designed compounds was performed using the SWISS ADME program. The acceptability of analogues based on Lipinski's rule of five which was essential to ensure drug like properties.

Lipinski rule of five:

- Molecular weight > 500
- C log P > 5,
- H-bond donors > 5
- H-bond acceptors > 10

All targeted derivatives (5a-5ab) followed the Lipnski rule of five. So it ensures that all the compounds have drug like properties such as adsorption, distribution, metabolism and elimination.

5.2.1 Experimental section

All the targeted derivatives were purified by recrystallization. All the targeted derivatives were characterized by spectroscopy techniques like IR, Mass spectroscopy, ^{1}H NMR, and ^{13}C NMR. I got appropriate spectral evidence in favor of structure elucidation.

5.3.1 In *vivo* biological evolution

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia. Compounds 5c (50.88±3.7), 5n (47.93±5.4), 5o (45.27±5.2), 5x (41.39±3.7), 5z (41.52±5.9) derivatives of sulfonylureas contain electronegative atoms (NO_2, F, Cl) on 4^{th} position of benzene ring was showing better % reduction of blood glucose level compare to other derivatives.

5.2 Achievements with respect to the objectives

Diabetes mellitus is chronic disorder increases day by day. Various micro and macrovascular complications are associated with it. With the help of literature review, docking studies, in *silico* toxicity studies, we have designed and synthesized novel hypoglycemic agents. In *vivo* biological evaluation gives results about blood sugar reduction in albino wistar rats.

5.3 Recommendation for future research

Novel hypoglycemic agents of various sulphonylureas/guanidine derivatives were successfully introduced into the therapy of diabetes mellitus. In future, further research is possible by taking various heterocycles like pyrrolidine, piperazine, oxazole, thiazole, pyrazine, oxadiazole with different substitutions. In vitro biological evaluation is furthermore scope of future research in an episode of antidiabetic therapy. HPLC method of novel derivatives can also develop.

5.4 Conclusion

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the current work, in the binding and interactions of sulphonylureas/guanidine derivatives with ATP-sensitive K^+ channel SUR1/Kir6.2 complexed with ATP and glibenclamide (PDB ID: 5TWV) was done. It was imported and studied using molecular docking. Most of the compounds have shown significant binding relations for same. It was observed that the benzene ring, Cl, F, NO_2 group, sulphonamide group is an important for binding interaction with receptor.

Compound 5c (-120.836), 5n (-124.972), 5f (-129.498), 5i (-120.41), 5p (-123.115) and 5z (-118.063) showed a better binding affinity in comparison with glibenclamide. However, the Compound 5c (50.88±3.7), 5n (47.93±5.4), 5o (45.27±5.2), 5x (41.39±3.7), 5z (41.52±5.9) shows better % reduction of blood glucose compares to other derivatives. So, the 4^{th} and 2^{nd} positions of derivatives substituted with F, NO_2, and Cl which was shown better result compare to unsubstituted or substituted with other derivatives.

CHAPTER 6

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APPENDIX A

Approval from CPCSEA & IAEC for in *vivo* biological evalution

PIPH	QUALITY RECORDS
At. & P.O. Limda, Tal. Waghodia, Dt. Vadodara.	QR-751-6.3 Project Protocols PIPH 03/16 CPCSEA 921/PO/EReBi/S/05/CPCSEA

Form B [per rule 8(a)]

APPLICATION FOR PERMISSION FOR ANIMAL EXPERIMENTS

Application to be submitted to sent either the CPCSEA (address in form A above) or Institutional Animal Ethical Committee (IAEC)

Part A

1. **Name & address of establishment** : Parul Institute of Pharmacy, Limda.

2. **Registration number & Date of registration** : CPCSEA REG NO: 921/PO/EReBi/S/05/CPCSEA

3. **Name, address & registration no. of breeder from whom animals will be acquired for experiments mentioned in parts B & C** : In-house

4. **Place where animals are presently kept** : Animal house of the Parul Institute of Pharmacy, Limda, Tal : Waghodia,Dist : Vadodara. State : Gujarat , India.

5. **Place where the experiment is to be performed (Please provide CPCSEA Reg.No)** : Parul Institute of pharmacy, Limda CPCSEA REG NO: 921/PO/EReBi/S/05/CPCSEA

6. **The date on which the experiment is to be commenced and the duration of Animal study experiment:** As soon as we get the approval from IAEC.

7. **Type of research involved (Basic / Educational/Regulatory)** : Basic

Signature

Date: 21/03/2016

Name of investigator: Mr. Ishan I Panchal
Place: Parul Institute of Pharmacy, Limda.

A LIST OF PUBLICATIONS DURING RESEARCH WORK

Sr.No	Author/S	Title of the Paper	Journal/Publication	Details 0f Journal (Vol., Issue, Page No.)
1	Ishan I Panchal, Dhrubo Jyoti Sen, Ashish D Patel, Umang Shah, Mehul Patel, Archana Navle, Vashisth Bhavsar	Molecular Docking, Synthesis And Biological Evaluation of Sulphonylureas/Guanidine Derivatives As Promising Antidiabetic Agent	Current drug discovery technologies (SJR, Scimago Journal & Country Rank 1.5), Scopus (Bentham Science)	Volume 14 , 2017 DOI: 10.2174/1570163814666171002102904
2	Ishan Panchal, Dhrubo Jyoti Sen, Umang Shah, Archana Navale	Structure Based Drug Designing, Scoring, And Synthesis Of Some Substituted Sulphonylureas/Guanidine - Based Derivatives As Hypoglycemic Agents	International Journal of Pharmacy And Pharmaceutical Sciences (Elsevier) (SJR Scimago Journal & Country Rank 0.51)	Int J Pharm Pharmceu Sci, Vol 9, Issue 12, 226-232
3.	Ishan I Panchal, Dhrubo Jyoti Sen, Ashish Shah, Ashish Patel, Vashisth Bhavsar	Molecular Docking And Synthesis of Some Substituted Sulphonylureas/Pyrrolidine - Based Derivatives as Hypoglycemic Agents	Journal of Chemical and Pharmaceutical Research. SJR (Elsevier) (SJR Scimago Journal & Country Rank 0.28)	J Chem Pharma Res, 2017, 9 (8): 164-172
4.	Ishan I. Panchal,Dhrubo Jyoti Sen, Samir K. Shah	Synthetic Approach Towards Some Substituted Sulphonylureas And Guanidine Derivatives As Hypoglycemic Agents	European Journal of Pharmaceutical and Medical Research	Euro J Phar Med Res, 2016,3 (3), 433-442
5.	Dhrubo Jyoti Sen, Ishan I. Panchal, Ashish D. Patel	Scifinder® As A Latest Tool In Innovative Research Within A New Dimension For Integrating Scientific Chemical Databases	European Journal of Pharmaceutical and Medical Research	Euro J Phar Med Res, 2016,3(11), 01-19
6.	Ishan I. Panchal, Dhrubo Jyoti Sen, Samir K. Shah	Novel Approach In Diabetes Mellitus: Say No To Sugar And Yes To Artificial Sweeteners	International Journal of Pharmaceutical Research and Bio-Science	Int J Pharm Res, 2014; Volume 3 (2): 770-784
7.	Ishan I. Panchal, Dhrubo Jyoti Sen, Bhavesh Prajapati,Samir K. Shah	Serendipity of Fluorine In Discovery And Development Of Antidiabetic Agents: A Bottleneck Systemic Review	World Journal of Pharmaceutical Sciences	World J Pharm Sci 2013; 1 (4): 168-175
8.	Ishan I. Panchal, Dhrubo Jyoti Sen, alkesh K. Patel,Samir K. Shah	Leptin Centered Therapy For Diabetes: Great Hope For Imminent	World Journal of Pharmacy and Pharmaceutical Sciences	World J Pharm And Pharma Sci 2013;3 (1), 795-806.